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AND THE ROTHAMSTED RESEARCH INSTITUTES BY

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**GROWTH AND DEVELOPMENT IN THE PIG,
WITH SPECIAL REFERENCE TO CARCASS
QUALITY CHARACTERS**

**PART IV. THE USE OF SAMPLE JOINTS AND OF CARCASS
MEASUREMENTS AS INDICES OF THE COMPOSITION
OF THE BACON PIG**

**PART V. THE BEARING OF THE MAIN PRINCIPLES
EMERGING UPON THE MANY PROBLEMS OF ANIMAL
PRODUCTION AND HUMAN DEVELOPMENT**

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PART IV. THE USE OF SAMPLE JOINTS AND OF CARCASS MEASUREMENTS AS INDICES OF THE COMPOSITION OF THE BACON PIG

(1) INTRODUCTION—METHODS, MATERIAL

THE composition of the animal body in terms of its major tissues is of fundamental importance in animal production. The data presented and discussed in the foregoing parts of this paper provide abundant evidence of this.¹ The accurate measurement, therefore, of the amount of bone, muscle and fat in the body is of prime importance to all interested in live-stock studies. Particularly is this the case in all technical studies

¹ This is the concluding part of work published in previous numbers of the *Journal*.

relating to meat production; all such must recognize the necessity for considering the purpose for which the animal is produced and must in consequence involve the examination of carcass quality. The animal nutritionalist, for example, was once able to stop short at the study of the chemical nature and digestibility of foodstuffs, and at the measurement of the efficiency of live-stock rations in terms of live-weight growth and by nutritional balance experiments. Since economic efficiency in production to-day involves the quality of the product, he must now consider also the effects of the materials and methods at his disposal upon the bodies of his animals.

This situation has become more widely recognized of recent years, especially in countries of supply for the large discriminating world markets. An increasing proportion of genetic, physiological and nutritional studies on all classes of meat animal are now carried to a stage permitting some examination of the carcasses produced. In all such work, the problem of measurement of the characters involved is a serious one. Chemical analysis of the bodies of the larger animals is technically difficult, laborious and expensive, and in consequence is possible in but few and isolated instances. It is subject to the disadvantage also that it yields results not very directly associated with many commercial aspects of carcass quality which affect the producer (Murray, 1922; Hammond, 1932a). The technique employed by American workers involving gross separation with the knife of fat, lean and bone, overcomes the latter disadvantage but still involves complete or partial destruction of the value of the carcass, and lacks the precision required in comparative slaughter work for the measurement of the small differences so frequent in experimental and practical treatments. Except on a limited scale the laboratory dissection technique used in this study is even more impracticable on economic grounds.

In consequence of this situation, much work to date has been dependent upon carcass measurements, either "external" or "internal" or both, as indices of composition and other quality differences (McMeekan, 1939). In the main, and particularly with the animal with which we are concerned, the measurements employed for this purpose have developed from the opinion and usage of the meat trade. Thus in the pig, where an optimum requirement of fat in the carcass is demanded, it has long been the regular commercial practice to assess the degree of fatness and consequent suitability for the particular trade concerned, by the depth of subcutaneous back fat at different points in the median line. Because of this, and since the practice is based on the logical assumption of a

reasonable correlation between such linear measurements and the amount of fat, investigators have employed back-fat measurements to assess the effect of experimental treatments upon fat development. Practically no serious attention, however, has been directed to the precise quantitative relationships between carcass measurements and the body tissues for which they are regarded as an index. One exception is the work of •Hankins & Ellis (1934) in respect to the character used in the illustration above. They established the reality of a high correlation between the mean back-fat thickness in the pig and the chemically determined amount of fat in the body. In this laboratory, and coincident with this study, Pálsson (1939-40) has recently investigated the value of a large number of different measurements as indices of bone, muscle and fat in the sheep. It is obviously important that such relationships should be worked out for each species if the great bulk of experimental and investigational studies practicable upon carcass quality problems are to be placed upon a scientific basis.

It must be emphasized that many of the measurements employed in carcass quality work can be justified on other than quantitative grounds. Many such measures may not necessarily bear any direct or important relationship to the amount of bone, muscle or fat in the body, but are nevertheless of considerable significance in the assessment of commercial quality of meat. Extreme examples of these are the colour of skin and flesh and the physical texture and colour of the fat. A thick belly is required in the bacon pig not because it is significantly related to one or other of the tissues concerned but because thickness in this region is essential to provide a saleable rasher of bacon. Other measures too, while having some quantitative significance, are more important because of their relationship to the proportions of the carcass. Thus a long leg is undesirable in any meat animal whatever the species, not merely because it is believed indicative of a high proportion of bone and is therefore wasteful, but because it is generally associated with a relatively thin cover of flesh and fat. Compared with a thick blocky leg with the same absolute amounts of meat, it provides a joint which dries out more in cooking and yields less attractive slices on carving. These characters affect the saleability of the product and must be taken into account. It is not our purpose to add to the already voluminous literature on the type of carcass required by specific markets which would involve detailed description of all such purely qualitative characters which vary in their significance from country to country and market to market. In the subsequent discussion, however, some reference will be made to

certain measures of importance in this connexion. As pointed out in our original definition of carcass quality (see General Introduction, Part I), the quantitative proportions of bone, muscle and fat in the body are universally the fundamental basis of quality, and it is with these that we are mainly concerned.

The data available from the experiment reported in Part III provide an excellent opportunity for examining the possibility of using sample joints as indices of total carcass composition and of testing the value of a large number of measurements for the same purpose. It is believed that the former is likely to provide a more precise basis in this connexion than the latter, and if so, and if the correlation between the composition of a suitable joint or joints is sufficiently high, would provide a practicable method capable of application to many types of experimental and investigational studies. The desirability of establishing the facts in respect of carcass measurement has been sufficiently emphasized above.

Complete dissection data, and carcass measurements obtained as described in the section dealing with technique and illustrated in Text-fig. 2 (Part I) are available for twenty bacon pigs of 200 lb. live weight. The wide extremes of nutritional treatment to which the animals have been subjected, and the consequent wide variation in type and in composition, provide a range which adds to the significance of the results of the present study. Thus the weight of bone ranges from 5.7 to 8.3 kg., muscle from 22.5 to 33.0 kg., and fat from 16.2 to 32.2 kg. The nature of the effects of the four treatments used (see Part III) involves a fairly uniform distribution in all three characters throughout the range (Appendix IV), and scatter diagrams show this to be the case.

The method of analysis used has been one of simple correlation on a basis of absolute values for both variates. Regression coefficients have been calculated in each case where the correlation coefficient is strongly significant. Where the value of the latter offers the opportunity of using the sample joint or measurement as a means of estimating composition with a reasonably high degree of accuracy, regression functions of the form $Y = y + b(x - \bar{x})$ have been developed, where b is the regression coefficient of y on x , and Y is the predicted value of y for each value of x (Fisher, 1934).

(2) THE LOIN AND LEG AS SAMPLE JOINTS

In the selection of possible anatomical joints capable of fulfilling the purpose we have in mind, several points must be taken into consideration. In the first place it is deemed advisable only to consider joints

which can be separated from the body with a high degree of accuracy. On these grounds, for example, the shoulder, the composition of which bears a close relationship to that of the carcass as a whole, is ruled out, for the absence of any well-defined cutting points offers little guarantee of satisfactory replication of results by different workers. In the second place, it seems desirable to utilize, if possible, the more valuable parts of the carcass, for if such are capable of providing the required estimate of the composition of the whole, they have the additional advantage of yielding also specific and accurate data upon regions of major importance. On these grounds, the relatively cheap cuts of the fore-end—head, neck and thorax—are unsuitable, while the loin and/or leg suggest themselves as most desirable. Both these, and particularly the loin, can be separated with a high degree of precision and have a still further advantage that their dissection offers less technical difficulty than many other joints.

Since we have shown that the different regions of the body and the different tissues of which it is composed develop at differential rates, it is somewhat anomalous to suggest that any one region should be capable of providing a perfect index of the whole. No one joint could be expected to yield satisfactory results for all three tissues. For this reason it is believed that the combination of two joints, the one relatively late developing and the other relatively early developing, offer greater opportunity of success. The loin and the leg fulfil all these conditions and have, therefore, been concentrated upon so far as this aspect of the work is concerned. The statistical relationships involved are set out in Tables 69 (B) (bone), 70 (muscle), and 71 (fat).

Table 69 (B). *Composition of loin and leg as an index of carcass composition*

A. Total weight of skeleton = dependent variate Y (g.)

Independent variate x (g.)	Correlation coefficient r	Regression coefficient $R_{v,x}$	Regression equation
Bone of loin	+ 0.9440	13.609	$Y = 13.609x + 1762$ g.
Bone of leg	+ 0.8969	8.614	$Y = 8.614x + 1328$ g.
Bone of loin and leg	+ 0.9444	5.614	$Y = 5.614x + 1125$ g.

In each case the data have been calculated for loin alone, leg alone, and for both joints together. In respect to bone the total weight of this tissue in all three cases is strongly and significantly correlated with the total weight of the skeleton, the respective coefficients approaching unity. For significance at the 1 % point r must have a value of 0.5614 and at the 5 % point of 0.4438 (Fisher, 1934).

The weight of bone in the loin shows a slightly higher figure for r

than does that of the leg, a result not unexpected in view of the relative differences in the rate of development of these bones. The bones of the limb are relatively earlier developing than the skeleton as a whole, while the vertebral column as a whole is fairly intermediate in development, though within this the lumbar units are relatively late. Combining the two joints a still higher correlation is obtained though this is not significantly so. Combination of the two joints can be expected to yield a higher correlation in all cases, not only because of the combination of an early and late developing unit but since it results in the comparison of a greater proportion with the whole.

Table 70. *Composition of loin and leg as an index of carcass composition*

B. Total weight of muscle = dependent variate Y (g.)

Independent variate x (g.)	Correlation coefficient r	Regression coefficient R_{y-x}	Regression equation.
Muscle of loin	+ 0.8782	11.931	$Y = 11.931x - 804$ g.
Muscle of leg (total)	+ 0.9711	7.435	$Y = 7.435x + 1992$ g.
Muscle of leg (round tibia)	+ 0.9177	49.87	$Y = 49.87x + 1309$ g.
Muscle of leg and loin (total)	+ 0.9765	4.983	$Y = 4.983x - 1454$ g.

It is apparent that the value of the respective coefficients permit the use of these joints alone or together to estimate the total weight of bone in the carcass with a high degree of accuracy, and regression equations for this purpose have been calculated in each case. The procedure is simple; if the weight of bone, obtained by dissection of the leg, amounts to x g., then the total weight of the skeleton is 8.614 times this figure + 1328 g.

In muscle an equally satisfactory situation exists; all correlations are strongly significant, and their closeness to unity indicates the reliability of the related regression equations as means of estimating the total weight of muscle in the carcass from the amount present in loin and/or leg. Note that the loin muscles show the lowest correlation and the total leg muscles the highest. An additional figure has been employed here in respect to leg muscles where the correlation between the weight of muscles surrounding the tibia and total carcass muscle has been calculated. The method of jointing the leg allows some degree of error for muscle with unskilled workmanship owing to a proportion of cut surface in the thigh muscles. The muscles round the tibia, however, form a complete and definite anatomical unit obtained by "separation", and their measurement is accordingly likely to be more precise. Though these show a lower correlation—probably due to their relatively early developing

character—than total leg muscles, this is sufficiently high to permit reliable estimation of muscle in carcass should risk of jointing errors be present.

In respect to fat, correlations have been established between the total fat in the carcass and both the total fat and the subcutaneous fat of the joints concerned. The reason for including the latter is twofold: correlations between total fat of leg and loin and that of the carcass though high were not considered high enough for the joints independently to give the standard of accuracy demanded for estimation purposes. Since subcutaneous fat forms by far the greater proportion of total fat in carcass, it seemed likely that estimates based on subcutaneous fat only might give more satisfactory results. Further, it is sometimes desired to estimate the fat only in the carcass, and if this could be obtained by dissecting out only the subcutaneous fat, which is much more easily removed, the work and expense involved would be considerably reduced. In respect to total weight of fat (subcutaneous plus intermuscular) neither the leg nor loin alone yielded particular strong correlations (Table 71), though a satisfactory figure is obtained with both joints taken together. The unexpected low figure for the loin is probably due to the high proportion of intermuscular fat in this joint relative to the low percentage in the total carcass.

Higher figures are obtained in all cases by working from subcutaneous fat only; this is specially the case in the loin where r increases from +0.8630 to +0.9434. The combined joints give an extremely satisfactory relationship with total fat with r equal to +0.9750.

Table 71. *Composition of loin and leg as an index of carcass composition*

C. Total weight of fat = dependent variate Y (g.)

Independent variate x (g.)	Correlation coefficient r	Regression coefficient R_{v-x}	Regression equation
Total fat of loin	+0.8630	5.713	$Y = 5.713x + 5630$ g.
Total fat of leg	+0.8815	15.804	$Y = 15.804x - 2063$ g.
Total fat of loin + leg	+0.9332	4.850	$Y = 4.850x + 462$ g.
Subcutaneous fat of loin	+0.9434	5.546	$Y = 5.546x + 9888$ g.
Subcutaneous fat of leg	+0.8920	17.361	$Y = 17.361x + 208$ g.
Subcutaneous fat of leg + loin	+0.9750	4.594	$Y = 4.594x + 5972$ g.

(3) CARCASS MEASUREMENTS AS INDICES OF BONE

In the foregoing sections of this paper, reference has frequently been made to the fact that linear measurements on the body of the animal are probably indicative more directly of the amount of bone than of

anything else. The reasons for this view are simply that all such measurements will depend largely upon the size of the animal, which is due in a very large measure to its "frame" or skeletal size. Though measurements made on the surface of the body are affected by the cover of muscle and fat they can be expected to provide but little guide to the relative proportions of these two tissues, while even their relationship to the two together is complicated by their major dependence upon length and shape of the bones. It will be noted, of course, that the very fact that such measures are affected to some extent by the flesh cover of the animal will reduce their reliability even as indices of skeletal development.

The majority of such measurements too are somewhat unsatisfactory owing to the very great technical difficulty in defining the positions or points between which they should be taken; in the live animal they are especially unsatisfactory for they are invariably affected by the position and movement of the animal.

The data presented in Table 72 support these views, for no external carcass measurement shows a sufficiently high correlation with the total weight of skeleton to enable the latter to be estimated with precision. Practically all, however, are strongly and significantly correlated with the total weight of skeleton. The particular measures investigated are those which can be measured on the carcass with a fairly high degree of accuracy in that their position is well defined by bone points. They are also those for which the highest correlation with skeletal weight has been found. Many other external measures were actually taken, and those reported are those which on graphical investigation gave promise of a reasonably high relationship with the amount of bone in the body.

Table 72. *Measurements as indices of carcass composition*

A. Total weight of bone in carcass = dependent variate Y (g.)

Measurement— independent variate x	Correlation coefficient r	Regression coefficient $R_{y,x}$	Regression equation
Length of fore trotter (mm.)	+0.7666	69.46	—
Length of fore arm (mm.)	+0.7190	39.02	—
Length of fore trotter + arm (mm.)	+0.8400	31.58	$Y = 31.58x - 2835$ g.
Length of leg (mm.)	+0.7938	17.27	$Y = 17.27x - 2947$ g.
Depth of chest (mm.)	+0.0418 (N.S.)	—	—
Weight of cannons (4) (g.)	+0.8964	34.60	$Y = 34.6x + 386$ g.

N.S. = Not significant.

It will be noted that the most satisfactory single measure is the length of hind leg (measured from symphysis pubis to the tip of the toes) with a correlation of +0.7938. The combination of the length of fore trotter with the length of forearm gives a still better relationship, r being equal

to +0.8400. A more reliable index from the point of view of estimation of the weight of bone in the carcass is obtained from the total weight of the cannon bones in grams which has the relatively high correlation with total skeletal weight of +0.9864. These are relatively easy to obtain and clean and offer an inexpensive practicable method for large-scale work on bone development in bacon-pig carcasses. Pálsson (1939-40) finds a similar high correlation between cannon-bone weight and skeletal weight in both lambs and hoggets. One measure is included which shows practically no correlation with skeletal weight—the depth of chest. The coefficient is not significant at +0.0418. Special attention is directed to this situation, for it is commonly believed in practice that pigs that are very deep through the chest have a relatively high proportion of bone in the fore-end of the carcass. The reason for the entire lack of relationship between this measure and bone is clear from our previous discussion of the effect of the different nutritional treatments upon the length, weight and thickness of the ribs (see Plate 22, Part III), and the relative effects of varying planes of nutrition upon bone and fat development respectively. Animals with the shortest and lightest ribs had the same depth through the chest as animals with the longest and heaviest ribs, because of their greater development of fat. This was the result of a heavy fat cover over and under the thorax which eliminated the difference in the actual skeletal chest depth. It will be clear that in any interpretation placed upon the chest depth measure in bacon pigs, due allowance will need to be made for the thickness of subcutaneous fat over the shoulder and under the sternum. The point is of special importance in view of the present defect in British bacon carcasses in respect to the chest depth measurement (Duckham, 1938; McMeekan, 1939).

(4) CARCASS MEASUREMENTS AS INDICES OF MUSCLE

The data reported in Table 73 cover for the most part "internal" carcass measurements obtained from the loin cut at the last rib. A few of the "external" measures yielding the most satisfactory correlations are included to illustrate the degree of the relationship between measurements of this type and the total muscle, while the position in respect to the weight of the psoas muscle has also been investigated.

The internal measures employed can be obtained with precision and are thus not subject to the disadvantage characteristic of external measurements. This applies also to the back-fat thickness and the fat measures of the loin cut (§ (5)).

Neither the "length of eye" A , nor the "depth of eye" B alone give satisfactory correlations, though the values are significant and indicate a general quantitative relationship with total carcass muscle. B gives a lower correlation than A , which is in line with their relative growth behaviour (see Parts I-III). The depth measure is relatively late developing, while muscle makes a large proportion of its growth relatively early in life.

Table 73. Measurements as indices of carcass composition

B. Total weight of muscle in carcass = dependent variate Y (g.)

Measurement— independent variate x	Correlation coefficient r	Regression coefficient $R_{y,x}$	Regression equation
Length of "Eye" A (mm.)	+ 0.6440	211.42	—
Depth of "Eye" B (mm.)	+ 0.5074	372.87	—
Shape index $B/A \times 100$ (mm.)	- 0.1720 (N.S.)	—	—
$A \times B$ (mm.)	+ 0.8414	5.33	$Y = 5.33x + 11125$ g.
$2A + B$ (mm.)	+ 0.9339	188.95	$Y = 188.95x - 10182$ g.
Length of carcass (cm.) + $2A + B$ (mm.)	+ 0.9288	172.77	$Y = 172.77x - 20178$ g.
Circumference at base of tail (mm.)	+ 0.4771	—	—
Length of fore trotter (mm.)	+ 0.8036	373.1	—
Circumference of forearm (mm.)	+ 0.4005 (N.S.)	—	—
Weight of psoas (g.)	+ 0.8143	35.28	$Y = 35.28x + 3371$

N.S. = Not significant.

The "Shape Index" ($B/A \times 100$) shows no significant correlation with total muscle weight, r being actually negative. This result is not surprising in view of its growth under varying planes of nutrition (Part III, § 8). Pálsson (1939-40) obtains a similar low correlation in lambs. Both the depth measure and the shape index have been extensively employed by different workers (Hammond, 1933, 1934, 1936b, 1937; Davidson *et al.* 1936; Hirzel, 1936; McMeekan, 1937; Pálsson, 1939-40) in studying the meat qualities of both pigs and sheep. The emphasis placed upon both of these measurements, however, finds explanation in the distinction previously discussed between quantitative and qualitative indices of carcass quality. A good depth of muscle and a high ratio of depth to length in this region of the meat carcass is essential from the commercial point of view. The loin is the most valuable part of the carcass, and it is the shape rather than the quantity of muscle in area which determines its suitability for high-quality and high-priced trade. Hammond (1933, 1936b) has discussed fully the details in this connexion.

Suitable combinations of the two measurements involved, however, give very satisfactory quantitative measures of total weight of muscle. Thus $2A + B$ (which gives more weight to A and is the total length of the eye muscle on both sides of the column plus its mean depth) shows a

correlation of +0.9339. By taking the total length of the carcass into account (from symphysis pubis to junction of first rib and sternum) on the grounds that the total muscle development will be related to the linear as well as cross-section surface of muscle, a similar high relationship is shown though this is no better than $2A + B$ alone. It is possible, however, that in animals showing more variation than these which do not differ widely in length may require its inclusion. Pálsson (1939-40) in lambs finds it helpful to include length to obtain high correlations for both muscle and fat.

$A \times B$, an approximation of the surface area of the "eye", shows a fairly high correlation with total weight of muscle. We have not made accurate measurement of the surface area of the eye muscle as employed by Sinclair (1935) and Woodman *et al.* (1936) because of the labour involved, the fact that it is not the percentage area of muscle and fat but the relative linear measurements of both on the bacon rasher which are of qualitative importance, and since we believed that as demonstrated above, the more practicable measurements would give a sufficiently high correlation on a quantitative basis.

A practical index of muscle employed in the Smithfield meat trade—the circumference of the tail base—does not indicate a very high relationship, being significant only at the 5 % point. This is not sufficiently high to provide a reliable index even for practical guidance. Of the other external carcass measurements, the circumference of the forearm shows a similar low correlation which is insignificant even at the 5 % point. A better figure is provided by the length of the fore trotter—also a fair index of weight of skeleton—due to the general relationship between bone and muscle growth (see Parts I-III). The relationship between the weight of the psoas muscle and the total muscle of the carcass is of special interest, for this muscle is readily available for experimental study on a large scale. It is invariably removed from bacon pigs in commercial practice and its weight can be easily recorded. As a complete muscle unit, and as a unit possessing the advantage of relative late development it has further advantages (see Part II). The psoas has been employed by Callow (1935b, 1936) and Woodman *et al.* (1936) in studying the effect of nutrition upon muscle growth; our results provide a quantitative basis for this practice, the correlation between psoas weight and total muscle weight being strongly significant with a value of +0.8143. Note, however, that this is not sufficiently high to enable the psoas to be employed with any high precision to estimate total muscle weight. The relationship is sufficiently high, however, for the psoas weight to merit

consideration as a measure of muscle development for stock improvement purposes. It will be noted from Table 73 that the only measures of value as muscle indices are those obtainable from examination of the cross-section of the body. Since cutting the carcass reduces its commercial value, many advanced registry schemes for pig improvement involving carcass examination are faced with financial difficulty in obtaining the necessary information on the scale necessary. For these, the psoas weight offers a practicable, if slightly less accurate substitute.

(5) CARCASS MEASUREMENTS AS INDICES OF FAT

The correlation and regression data in respect to a large number of fat measurements with total fat (subcutaneous and intermuscular) are set out in Table 74.

Table 74. *Measurements as indices of carcass composition*

C. Total weight of fat in carcass = dependent variate Y (g.)

Measurement— independent variate x	Correlation coefficient r	Regression coefficient $R_{v,x}$	Regression equation
Shoulder fat (mm.)	+0.8709	636.0	$Y = 636.0x - 2755$ g.
Loin fat (mm.)	+0.9312	594.7	$Y = 594.7x + 10988$ g.
Mean shoulder + loin (mm.)	+0.8764	614.9	$Y = 614.9x + 4369$ g.
Rump fat (mean) (mm.)	+0.9494	495.4	$Y = 495.4x + 9524$ g.
Mean back fat (mm.)	+0.9552	616.3	$Y = 616.3x + 4897$ g.
Fat at C (mm.)	+0.9663	556.3	$Y = 556.3x + 11973$ g.
Fat at H (mm.)	+0.9492	426.4	$Y = 426.4x + 10587$ g.
Weight kidney and leaf fat (g.)	+0.8020	11.11	$Y = 11.11x + 6933$ g.

The thickness of shoulder fat shows the lowest correlation, r being equal to +0.8709. As one proceeds posteriorly along the back line the value of the correlation increases. The thickness of fat at its thinnest point (loin) gives the relatively high value of +0.9312, while the thickness of the rump (mean of three measures) shows the highest correlation of these three. Combination of the thickest and thinnest fat, due to the predominating influence of the former, gives a relatively low correlation. The mean back-fat (mean of shoulder, loin and rump) thickness shows the highest correlation of all measures along the back line, being exceeded only by the thickness of fat at C on the loin cut. The coefficient for fat at H on this surface is at a similarly high level.

The relationships shown by all these measures are consistent with their respective behaviour with growth. The weight of fat in the body, as a late-developing tissue, is correlated best with measurements taken on the relatively late-developing regions. The higher coefficient between fat of loin than fat of shoulder and the total carcass fat is explainable on this basis. The position supports the suggestion of Hammond & Murray

(1937) that due to the relative development of these two measures with increased weight of carcass, the loin-fat thickness, being the later developing character, should provide the more efficient index of fatness in the pig. Rijssenbeek (1936) by comparison of shoulder-fat thickness with the percentage of back fat, obtained by gross separation) comes to the same conclusion.

• The general high nature of all the correlations, most of which closely approach unity, provides a measure of the value of the resulting regression equations as means of estimating the carcass fat on a basis of measurements alone. The correlations are higher than those (+0.84 for mean back fat) obtained by Hankins & Ellis (1934) between measures at similar positions and carcass fat. There are several probable reasons for this. They estimated the total fat in the carcass by chemical analysis and calculated the correlations between measurements and the percentage of fat. While percentage fat is a sound basis for the calculation of such relationships when the weight upon which it is based is uniform for all pigs, it is not sound where, as was the case with their data, carcass weight varied considerably. It is possible to have two animals of different body weight with the same percentage fat and consequently different absolute amounts and measurements. This situation naturally weakens a correlation calculated on a percentage basis. Our data showing the variation in the percentage fat in fat tissue with age and treatment (Parts I-III) provide a further reason for the difference.

Our results do not support the general criticisms of Woodman *et al.* (1936) in respect to back-fat thicknesses as measures of the general state of fatness in the pig. This is explainable on the grounds that they employed the area of fat in typical rashers as an index of total fatness of the carcass and correlated the different measures with the percentage results obtained. These two points alone would account for much of the weakness of the correlations reported. In addition, the correlation of measurements in one part with those in another part when the rate of development of these varies, would further weaken the relationships; this also might explain the lower correlations obtained for gilts than hogs. We do not find any difference between the sexes in the strength of the correlation between back-fat measures and total weight of fat.

(6) DISCUSSION

While so far as our material is concerned the results of the foregoing analysis have established beyond doubt the suitability of certain parts of the body for the purpose of estimating with accuracy the anatomical

composition of the whole, an investigation of this nature invites criticism as to how far the results obtained are applicable to animals of different origin. Similarly, while we have established the reality or otherwise of relationships hitherto mainly presumed to exist between carcass measurements and composition, the same question may be reasonably asked in respect to the resulting regression functions. The different breeds of pigs may vary widely in the characters under discussion, while different types and strains within any one breed may show equally wide differences.

In the first place it must be emphasized that our major objective has been to establish the principles involved. We have sought to demonstrate the strength or weakness of certain relationships which, because of their biological basis, must logically apply in principle though not necessarily in mathematical detail to all pigs whatever their origin. We have been concerned, however, with the precise statistical expression of the relationships under discussion with the definite purpose of application to future studies on the inbred strain of pigs used in this investigation. Many studies and experiments on future generations of these animals not previously practicable on grounds of labour, expense and uncertainty as to technique will now be possible.

At the same time we believe that there are reasonable grounds for suggesting that within limits the data presented are applicable to other than the specific animals from which they have been derived. There is much evidence to support the theory of Hammond (1935, 1937) that breed, type and strain differences in body proportions and composition lie essentially in differential rates of development of these characters. The experimental studies reported in this paper have provided conclusive evidence of the tremendous modifying influence of nutrition on these same characters, and we have also shown that the nature of these effects is explainable on the same grounds as are breed and type differences. By extreme nutritional treatments upon the inbred animals providing the material for this study, we have, in effect, produced differences in proportions and composition comparable in range and nature to the differences existing between pigs of varying breed and type. Our individuals cover an exceptionally wide range so far as their composition differences are concerned—a range which receives added emphasis from the fact that they are of the same body weight. The spread of nearly 100 % in the amount of fat, and comparable wide differences in the more stable bone and muscle tissues, are not likely to be exceeded under normal conditions in pigs of the same weight in the Large White or other bacon-type breeds. Even within the earlier maturing pork-type breeds

the range and nature of our data and the strength of the correlations are such that these will hold for such animals also, though it is probable that the regression functions will require modification. That is, there is every reason to suppose that an equally strong correlation exists between mean back-fat thickness and the total weight of fat in an extreme pork type such as the Berkshire, as for our pigs; though due to a different distribution of fat in the body as a result of differential development, the regression relationship may be different. For the reasons given above, however, even in such an extreme case the difference is not likely to be great. Pálsson (1939-40), working with lamb carcasses ranging from an extreme unimproved breed (Iceland) to a highly improved early developing breed (Southdown), obtained correlations as strong as ours between measurements, sample joints and composition.

Any application of our data to a different strain of pigs will naturally require caution. It is essential that the animals be of the same body weight and that their measurements fall within the range covered by our data; our regression functions will not necessarily apply outside this range. It would also be desirable, particularly if application to breeds other than the Large White were contemplated and prior to dependence on sample joints or measurements, to dissect completely a small number of individuals, preferably varying in type, to see how close they fit the regression lines concerned. Providing the first two precautions are taken, however, the regressions as they stand could be employed without much risk for large scale practical investigations on bacon-type breeds, providing a very high standard of accuracy is not essential.

It must be made clear that in advancing the above opinions we are not advocating any such application but rather have aimed at discussing the possibilities should this be contemplated. For the vast bulk of the type of investigations made on carcass quality problems in the pig the use of carcass measurements suffice, and in providing a scientific basis for their use, this study has its greatest practical value.

(7) SUMMARY

1. The accurate measurement of the amount of bone, muscle and fat in the bodies of meat animals is of considerable importance in all technical studies relating to carcass quality. Chemical analysis and laboratory dissection of complete animals are associated with disadvantages which preclude their extensive use. Material derived from nutritional experiments has provided the opportunity for statistical investigation of the

possibility of using sample joints and carcass measurements as indices of the composition of bacon pigs of 200 lb. live weight. The extent and nature of the variation in the composition of the pigs concerned adds to the significance of the results obtained.

2. The total weight of bone, muscle and fat in the bacon-pig carcass can be estimated with a high degree of accuracy from the respective weights of these tissues in either the loin or the leg. The combination of these two joints provide even higher correlations in each case than either one alone. In all cases the correlation coefficients approach unity and are strongly significant at the 1 % point. For the combined joints the values of r for bone, muscle and fat respectively are +0.9444, +0.9765 and +0.9750. Regression functions have been developed for the purpose of estimating carcass composition from these joints.

3. By establishing the existence of correlations hitherto only presumed between certain carcass measurements and composition, the use of these in the study of carcass quality in the bacon pig has been justified on scientific grounds. At the same time it is pointed out that the use of some carcass measures not necessarily bearing any quantitative relationship with bone, muscle, or fat, can be justified on purely qualitative grounds.

4. "External" carcass measurements are shown to be mainly indicative of the degree of skeletal development. The fact that they are influenced by the flesh cover of the animal reduces their value even for this purpose. Of the linear measurements the "length of hind leg" and the combined "length of fore trotter and forearm" provide the best indices of the amount of bone in the carcass, but the value of the correlation coefficients concerned, though strongly significant, is too low to allow estimation with any high degree of accuracy. The combined weight of the cannon bones provides a better index of total weight of skeleton than any linear measurement. The depth of chest bears no relationship to the bone in carcass due to the differential response of the tissues to varying planes of nutrition.

5. Suitable combinations of internal linear measurements of muscle obtained on the cross-section at the junction of thorax and loin are highly correlated with the weight of muscle in the carcass and are capable of providing a reliable basis for its estimation. The "eye"-muscle measurements, $2A + B$ with a correlation coefficient of +0.9339 shows up best in this respect. Neither "length of 'eye' A", or "depth B" are suitable alone for this purpose though both are significantly correlated with total muscle weight. Linear external carcass measurements are not strongly

correlated with total muscle. The relationship of the weight of the psoas muscle to total muscle is sufficiently strong to justify its use as an index of muscle development for certain classes of investigational and experimental work.

6. Correlation between various measures of the thickness of back fat and the total weight of fat in the carcass are particularly strong, and for the most part closely approach unity. Fat at the shoulder gives the weakest, and at rump (mean of three) the strongest coefficient of the single measures, but the latter is exceeded by the mean back-fat thickness (mean of five) with r equal to +0.9552. Fat measurements obtained from the loin cut similarly show high correlations with total weight of fat. That of fat at C attains the maximum value of any fat measure with r equal to +0.9665.

7. It is contended that the relationships established will, because of their biological basis, apply in principle, though not necessarily with the same mathematical constants, to all pigs of the same body weight, whatever their origin, breed or type. Any application of the regression functions developed in respect to both sample joints and measurements, to pigs other than the strain from which these have been derived requires considerable caution. With pigs of the bacon type breeds, reasonably accurate results should be obtained for practical purposes, providing the pigs are of 200 lb. live weight and their carcass measurements come within the range covered by our data. For most types of comparative slaughter work, however, carcass measurements alone provide a sufficiently sound basis on both practical and scientific grounds.

PART V. THE BEARING OF THE MAIN PRINCIPLES EMERGING UPON THE MANY PROBLEMS OF ANIMAL PRODUCTION AND HUMAN DEVELOPMENT

In attempting to review in general terms the results of these studies, we find that we are left with a deep appreciation of two outstanding characters of the animal body. These are its plastic and its resilient nature. On the one hand, its form and its tissues can be moulded and shaped to a remarkable degree by the influence of the nutritional environment. On the other, it possesses an amazing recuperative capacity; its tissues are capable, in certain cases and circumstances, not only of tolerating extremes of environmental treatment, but under specific conditions, can largely recover from the limiting effects of unfavourable environments. The animal is not an isolate in a neutral environment but a living organism

dependent upon, and responsive to, the environment in which it finds itself for the expression of its inherent capabilities. That there is a limit both to the plasticity and the resiliency of the body is implicit in the nature of the relationship between environment and growth. The major modifications in form and anatomical composition do not occur as isolated effects but rather as orderly changes spread over a number of correlated parts and originating in "some deep-seated rhythm of growth":

In approaching our problem, which in its most limited form has been the investigation of how far the conformation and composition of the pig can be modified and controlled by appropriate control of the nutritional environment, we have taken advantage of the phenomenon common to animals of all species; the phenomenon of differential growth of the constituent parts of the body. The resulting demonstration, that the profound influence of nutrition upon the development of the animal is fundamentally dependent upon the fact of differential growth, provides strong theoretical grounds for suggesting that the principles emerging are applicable also to species other than the pig. The fact that characters, which have been experimentally studied in sheep, cattle and horses, also respond differentially to nutrition adds weight to this suggestion.

That the environment and particularly the nutrition is capable of producing marked effects upon the form of his animals has long been realized by the breeder of live stock, and the history of breed improvement provides abundant evidence of the use which he has made of its power. He has not always been consciously aware of the fundamental basis of the association, however, nor has he known the precise nature of the effects upon many characters of his animals. In this detailed analysis we have tried both to provide him with this basis and to fill in some of the gaps in existing knowledge by which his control over the farm animal may become more complete and effective. The concept of the shape of the growth curve as a factor of considerable importance in animal production is a somewhat new angle the appreciation of which can do much in this direction.

The four types of growth curve which have formed the basis of this study are characteristic of the major differences which occur in the growth of animals in every country in consequence of differences in the efficiency of individual husbandry. In one or more cases they are typical also of the productive methods of every country as a result of natural variations in the food supply. Though nutrition alone has been employed in this work, other environmental factors, such as parasites, temperature, light and exercise (in search for food), are similarly capable of affecting the rate

of growth of the animal by quantitative control of the nutritive energy available. We have also shown that wide differences in the qualitative plane of nutrition, even to variations far greater than normally met with in the field in the ratio of protein to carbohydrate are covered in principle by our results. Only in respect to the influence of essential qualitative factors, such as minerals and vitamins, do our results not apply. It must be noted, however, that the evidence available from a great bulk of experimental work show that these also are capable of imposing specific limitations upon growth.

Domestic animals are kept in an artificial environment created by man. The problem of how the environment be arranged to fit the species and the breed and the species and breed selected to suit the environment is one of primary importance in animal production. On its solution rests the success, or otherwise, of a live-stock industry. By studying the relationship between the growth of the animal and the major environmental modifications to which it is likely to be subjected, we have endeavoured to obtain such knowledge of the principles operative that, in general terms at least, the results of particular combinations can be predicted, or recommendations for the attainment, of specific results formulated for a wide set of circumstances. The practical applications are thus manifold, as will be obvious to any with knowledge of live-stock problems.

The evidence afforded during the course of the study on the relationship between the influence of nutrition and the differences in form and composition within and between species, adds to the scope in this connexion. That is, a particular result may be attained either by imposing a nutritional environment in the direction necessary and/or by a change in the breed or strain to an earlier or later developing type as the case may be. The possibilities in this connexion have been ably dealt with from the animal husbandry point of view by Hammond (1936c) in discussing the stratification which exists in time and space in animal production.

While the growth approach has thus provided a fundamental basis for the interpretation of differences in the meat-producing qualities of the animal and has permitted the enunciation of general principles relating to the influence of environment as a directive force in the production of farm live stock, the results obtained are suggestive also in respect to their bearing upon related problems of animal development and in indicating the lines along which further investigations might profitably proceed. Our studies, for example, have stopped short at an

immature stage in development, and a question which attains prominence from the nature of the effects is that of the permanence of environmental influences upon the animal body. This problem is an important one to breeders of all classes of animal. Does a long-continued low level of nutrition or a permanently inadequate level stunt the maturity size of the individual and alter its proportions? Or conversely, does the imposition of extremely high levels of nutrition in the search for more efficient productivity in all its aspects, impair in any way the ability of the animal to maintain a long productive life? What are its effects upon longevity and upon resistance to disease? Again, what are the effects from both these points of view of the alternating seasonal periods of high and low nutrition characteristic of many countries where man's control over the environment of the animal is relatively ineffective? Further, and even more important from the point of view of breed improvement, should environmental influences produce a permanent effect, what are the repercussions upon the subsequent generations? In other words, what are the genetic implications of the environmental influences with which we have been concerned?

While our investigations do not pretend to provide any answer to these points, and while our main emphasis is on the necessity for prosecuting planned research along such lines, the results obtained have some bearing upon them. Reference has already been made as to the probability of the permanence of nutritional effects and the fact that while we have been impressed with what we have termed the resiliency of the animal body, our present data do suggest that the mature size and form of the animal may be permanently affected by prolonged and severe undernutrition. Confirmation is wanting and can be obtained only by carrying the experiment to maturity.¹ Permanency in effect is implicit in our interpretation of the nature of the response of body tissues to nutrition; its dependence upon the differential growth of the different tissues implies that continued long enough and at a sufficiently severe level, undernutrition must permanently stunt the growth of the earlier developing parts. For the same reason it must prevent the full development of late-developing units of the body. The relationship of experimental evidence in respect to other animals in this connexion and the general support of the hypothesis has already been reviewed.

The general evidence available from the field of animal husbandry is similarly in support of a permanency in effect. We see this in the

¹ Since this was written some evidence for the permanence of nutrition effects on body proportions in these pigs has been obtained (McMeekan & Hammond, 1940).

degeneration to a form approaching the unimproved animal in the Shorthorn cattle imported to the Argentine in consequence of which continued importation of English cattle is made. The difficulty is being overcome by the establishment of special bull-producing farms where a higher level of nutrition permits the highly developed improved form to be retained. It is evident also in the permanently increased size and coarseness of Jersey cattle in New Zealand, in consequence of which continued importations from Jersey Island are made by breeders in an effort to retain the true Jersey type. The wild pigs of the New Zealand bush, which are escaped domestic breeds of English origin, are hardly distinguished in their mature form from the true wild pig of Europe. Hammond (1936c) has provided numerous examples of this type.

As to the converse, it is generally believed that nutrition which permits maximum growth and development is desirable in terms of the life span as a whole. This view, however, is being challenged by the experimental work of McCay *et al.* (1935), which suggests that animals made to grow slowly by calorie restriction have a longer breeding productive life than those growing very rapidly under *ad libitum* feeding. It is also becoming a matter of doubt in the minds of many animal husbandrymen who view with dismay the apparent increased incidence of disease accompanying increased efficiency of production as measured in terms of weight, size, milking capacity, and egg-laying ability. While we have no evidence in any way suggestive of such an association, it is to be emphasized that in the long run, the productive lifetime performance is the final test of success in methods of rearing many of our animals. In man we are interested in a healthy life in which the infirmities of old age are postponed as long as possible. While one may doubt that the way to achieve this desirable objective is to starve slowly to death, it must be admitted that it is not possible to say with certainty that rapid growth is wholly beneficial until influence upon lifetime production over several generations has been examined as thoroughly as its effects during the growth period itself. Equally important as these two extremes are the effects of alternating periods of good and poor nutrition. Where these are operative during the early growing period—the period of productive life of the animal killed for meat—we can from our results predict with reasonable certainty the nature and general trend of the effects, but the cumulative results upon the mature form and over many generations must, as with the cases mentioned above, remain for future experimentation and investigation to clear up. It can be said from our work, however, that if these seasonal variations are mainly quantitative in

character, and providing they are not unduly severe or prolonged in their application, the flexibility of the animal will permit a considerable degree of recovery. The extent of this will depend upon the period of life over which the conditions are operative in addition to the two factors noted, and will for the same reason become less as age advances. The combination of qualitative limiting factors under such condition will in general theory still further increase the risk of permanent effects.

While it is with diffidence that we suggest the facts arising from this study have a bearing on problems of human development, the same reasons as governing their application in principle to animals other than the pig are surely operative also in the case of man. The rat has contributed largely to human welfare, and it should not be too much to suggest that the pig may also be able to add his quota of information. The attention directed of late in this country to undernutrition amongst a large section of the population has emphasized the necessity for reliable data upon the precise effects of quantitative and qualitative deficiencies in human diets. Several aspects of our work should prove of interest in this connexion. The relative development of the body under high and low quantitative levels, the importance of the shape of the growth curve, the relative unimportance of wide variations in the qualitative relationship of protein to carbohydrate providing adequate protein is available for growth, and the capacity of all tissues to recover from relatively severe degrees of undernourishment, must all, in some degree, be applicable to man. In human welfare, as well as in animal production, the question of the permanency of effects are also of great significance. It would also be of interest to know the age after which the capacity for complete recovery from retardation, of children reared under slum conditions, is no longer possible.

Recognition of the controlling influence of environment in the development of the animal body and of the permanent nature of its effects upon the mature form invites consideration of its relationship to the hereditary factors believed to set the limit to the expression of all characters. With genetically comparable material we have seen that high nutrition has produced effects comparable to those which have occurred during the evolutionary improvement of a breed. On the other hand, severe low nutrition has produced animals comparable with the unimproved and primitive form. Are these effects likely to be transmitted to subsequent generations? In this connexion the views of Hammond (1935) challenge attention. He advances the view that while the science of genetics explains the mechanism of inheritance it does not satis-

factorily account for the mechanism of evolution, and suggests that the evolution of present-day breeds of live stock is capable of explanation only on the hypothesis that environment exercises a specific directive force, and that under its stimulus the animal is capable of developing genes by which the induced characters are transmitted to subsequent generations. As he points out, the alternative is the theory that all characters capable of expression already exist and that environment merely controls the extent to which they are developed, all improvement consisting in selective assortment in the process of reproduction. This, supplemented by the theory of mutations, is the present generally accepted cause of variation in animals. There is no evidence that any of the characters concerned in meat production have arisen as mutations. Mutations are in the vast majority of cases recessives occurring haphazardly without any relation to environment, and for the most part are responsible either for defects or for economically unimportant characters. Acceptance of the view that all genes are in existence implies that the "amoeba has the capacity for milk production".

While it is neither our purpose nor function to argue the pros and cons of these fundamentally divergent views, the implications of the theory of developed genes is of supreme importance in breed improvement, in view of our demonstration of the nature and extent of nutritional influences. We have, in effect, shown that the physiology of the animal body can greatly be influenced by the nutritional environment. We know that the physiology of to-day is the developmental history of to-morrow, and may, therefore, reasonably ask whether the process by which the specialized function of the gene and chromosome has been produced by the cell during the course of evolution may not still be going on to-day under the directive influence of environmental stimuli. Whether the physiology of the animal can affect the germ-plasm must remain a matter for speculation. Whatever the case it is clear that we are imposing upon the geneticist a heavy burden in asking him to devote his attention to the study of animal characters of the type with which we have been dealing, characters which are as responsive to the environment as we have shown them to be. Any attempt to obtain the measurements necessary for such study must obviously be obtained from animals reared under optimum conditions for the development of the characters concerned. Since such conditions are difficult, if not impossible, of attainment on the scale necessary in the vast majority of circumstances, it is not surprising that the science of genetics has failed to live up to the promise of Mendelism in respect to the practice of animal breeding.

May we conclude with a quotation from Hammond (1935) to whom this work as a whole owes so much:

"The further development of these commercial qualities in our animals depends, like the 'civilization qualities' in man, on the creation of a better environment for the development of the characters concerned."

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APPENDIX IV

Composition of carcass at 200 lb.—high-protein series

	High-High						Low-Low					
	♂	♂	♂	♀	♀	♀	♂	♂	♂	♀	♀	♀
Head (g.)	83	70	106	Mean	84	74	99	71	107	Mean	80	82
Total weight	4937	5244	4944	5042	4571	4948	4760	5552	5492	5000	5348	5732
Bones:												
Tongue	10	9	9	9	344	344	10	10	9	11	10-3	12
Lower jaw	325	363	343	344	1022	1151	414	379	425	440	444-3	12.5
Skull	968	1056	932	985	1087	1252	1164	1170	1195-3	1307	510	524
Total	1303	1428	1284	1338	1376	1575	1476	1729	1600	1621	1650	1339
Muscle												
Subcutaneous	785	774	806	789	501	566	534	329	428	171	309	331
Intermuscular	369	490	447	435	328	302	315	318	299	295	304	294
Total	1154	1264	1253	1224	829	868	849	647	727	466	613	584
Fat:												
Tongue	135	161	175	157	150	161	156	247	192	213	217	227
Brain	98	101	99	99	95	97	96	114	122	112	116	114
Eyes	11	11	11	11	11	12	11	14	14	13	14	14
Skin	305	256	237	266	249	229	239	261	166	216	305	278
Ears	322	335	305	321	320	384	352	410	350	341	463	544
Tendon and waste	444	419	312	392	373	477	425	567	777	728	691	504
Glands	37	122	108	89	63	71	67	70	81	55	69	656
Loss in dissection	180	187	114	160	81	93	87	131	96	83	103	77

APPENDIX IV (*continued*)

Composition of carcass at 200 lb.—high-protein series

	High-High				Low-Low				♀	♀	♀
	♂	♂	♀	♀	♂	♂	♀	♀			
Neck (g.)	83	70	106	Mean	84	74	90	Mean	75	76	75.5
Total weight	5786	5674	5013	5491	5870	6571	6221	6341	6439	6011	6264
Bones:											
Atlas	58	60	57	58.3	63	65.5	77	84	75	75	75.5
Axis	52	48	51	50.4	52	53	52.5	65	60	59	59.5
Cervical	192	190	203	195.0	235	227	231	230	246	228	234.7
Total	302	298	311	304	355	343	349	367	395	363	375
Muscle	2170	2064	2033	2089	2458	2284	2586	2969	3133	3011	2910
Fat:											
Subcutaneous	2003	1890	1732	1559	2094	1826.5	1384	1390	1164	1313	1189
Intermuscular	791	1024	861	892	982	1213	1097.5	1223	1139	867	1076
Total	2794	2827	2251	2624	3201	2924	2607	2529	2031	2389	2149
Skin	296	282	227	268	328	327	328	257	359	322	313
Tendon, glands, etc.	84	84	76	81	99	123	111	107	162	85	118
Loss in dissection	140	209	115	155	59	187	123	71	25	77	58
High-Low											
Neck (g.)	85	72	98	Mean	67	103	89	♂	♀	♀	♀
Total weight	6966	5946	7005	6639	6987	6705	6846	6634	6962	6798	6372
Bones:											
Atlas	57	58	71	62	69	75	72	52	46	49	58
Axis	50	45	58	51	57	58	57	44	40	42	46
Cervical	202	197	248	216	238	230	234	186	157	171	196
Total	309	300	377	329	364	363	363	282	243	262	302
Muscle	2391	2517	2828	2579	3021	3204	3113	1963	2123	2043	2108
Fat:											
Subcutaneous	2147	1744	1861	1917	1833	1376	1605	2320	2756	2538	2006
Intermuscular	1597	991	1393	1327	1103	1032	1067	1444	1536	1490	1420
Total	3744	2735	3254	3244	2936	2408	2672	3764	4292	4028	3426
Skin	300	255	314	290	330	492	411	286	274	280	272
Tendon, glands, etc.	149	73	93	105	146	108	127	93	40	67	73
Loss in dissection	73	66	139	92	190	130	160	246	+10	118	191

APPENDIX IV (continued)

Composition of carcass at 200 lb.—high-protein series

	High-High						Low-Low					
	♂			♀			♂			♀		
	♂	♂	♂	♀	♀	♀	♂	♂	♂	♀	♀	♀
Left shoulder g.	83	70	106	84	74	99	71	107	107	80	82	82
Total weight	5680	5660	6070	5804	5380	5490	5840	6037	5699	5550	5223	5387
Bones:												
Scapula	148	167	150	155	167	170	169	163	156	160	165	165.5
Humerus	238	246	236	240	235	254	245	285	280	285	270	289
Radius-ulna	160	168	163	164	155	166	160	183	177	175	178	185
Carpels	39	42	40	40	37	35	36	43	47	42	44	39
Cannons	47	46	50	48	45	47	45	54	50	52.3	46	48
Splints	14	14	13	14	13	13	13	14	14	15	14.3	13
Dew claws (2)	11.6	14.1	11.3	12.3	12.7	11.8	12.3	12.0	14.7	11.9	12.9	12.3
Pasterns (2)	19.4	22.3	21.9	21.2	20.1	21.1	20.6	22.7	24.1	21.7	22.8	21.7
Coronets (2)	12.0	13.8	12.4	12.7	12.8	12.3	12.6	13.2	15.2	13.1	13.8	13.0
Pedals (2)	7.4	8.0	7.8	7.7	7.7	7.7	7.7	8.1	10.2	8.2	8.8	8.4
Naviculars (2)	0.6	0.6	0.6	0.6	0.8	0.8	0.8	1.0	1.1	0.7	0.9	1.1
Seamoids (2)	5.1	6.5	5.6	5.7	5.6	6.4	6.0	6.1	7.1	6.8	6.7	4.5
Total bones	702	748	712	721	712	745	729	809	805	785	800	783
Muscle:												
Shoulder	2302	2324	2643	2423	2536	2401	2469	2840	2840	2911	2864	2807
Arm	232	240	256	243	262	246	254	338	319	297	318	310
Round cannon	8	8	17	11	12	14	13	19	18	18	18	19
Total muscle	2542	2572	2916	2677	2810	2661	2736	3197	3177	3226	3200	3136
Fat:												
Subcutaneous	1402	1308	1307	1339	967	1047	1007	888	1157	768	938	719
Intermuscular (shoulder)	448	408	547	468	387	487	437	438	385	318	380	312
Intermuscular (arm)	42	29	28	33	22	31	27	32	27	33	31	27
Total fat	1892	1745	1882	1840	1376	1565	1471	1358	1569	1119	1349	1058
Skin	335	273	317	308	300	297	298	263	269	323	285	392
Tendon, glands, etc.	159	155	136	150	148	165	156	189	183	191	188	174
Loss in dissection	50	167	107	108	14	57	35	24	34	55	37	27

		High-High						Low-Low					
		♂			♀			♂			♀		
		♂	♂	♂	♀	♀	♀	♂	♂	♂	♀	♀	♀
Right shoulder	83	70	106	Mean	84	74	Mean	99	71	107	Mean	80	82
♂													
Total weight	5500	5560	5460	5507	5270	5350	5310	5742	5917	5675	5778	5590	5518
Bones:													
Scapula	147	154	161	154	169	165	167	166	167	162	165	157	163
Humerus	237	247	240	241	237	252	244	291	288	282	287	279	288
Radius-ulna	166	172	168	169	154	167	160	183	177	177	179	170	186
Carpals	40	43	39	41	37	36	37	44	48	41	44	39	38
Cannons	46	48	50	48	44	46	45	52	54	49	52	47	48
Splints	15	13	15	14	13	13	13	14	15	15	15	15	14
Dew claws (2)	11.1	12.5	12.3	12.0	12.5	9.8	11.2	13.6	13.2	13.4	13.4	12.8	11.8
Pasterns (2)	19.0	21.7	22.1	20.9	20.0	18.8	19.4	23.8	24.1	22.1	23.3	21.8	21.5
Coronets (2)	11.4	13.0	13.3	12.9	12.6	10.8	11.7	14.6	15.2	13.5	14.4	13.0	12.9
Pedals (2)	7.2	7.6	8.7	7.8	7.6	5.8	6.7	9.1	10.2	8.9	9.4	8.6	7.5
Naviculars (2)	0.7	0.6	0.6	0.6	0.7	0.6	0.7	1.1	1.1	0.7	1.0	0.8	0.9
Seamoids (2)	5.1	5.4	5.6	5.4	5.2	4.7	5.0	6.4	7.0	6.8	6.7	5.7	5.8
Total bones	706	738	736	727	713	730	721	819	820	791	810	770	797
Muscle:													
Shoulder	2187	2387	2310	2295	2439	2470	2455	2805	2827	2922	2851	2824	2786
Arm	226	252	259	246	277	245	261	336	328	306	323	314	291
Round cannon	12	12	16	13	12	18	15	20	17	17	18	18	17
Total muscle	2425	2651	2585	2554	2728	2733	2731	3161	3172	3245	3192	3156	3094
Fat:													
Subcutaneous	1297	1170	1081	1182	939	917	928	803	952	744	833	693	625
Intermuscular	407	424	448	426	316	501	409	435	395	354	395	370	398
(shoulder)													
Intermuscular	40	34	25	33	24	26	25	31	26	23	27	25	24
(arm)													
Total fat	1744	1628	1554	1641	1279	1444	1362	1269	1373	1121	1255	1088	1047
Skin	306	253	295	285	283	251	267	269	305	293	355	335	345
Tendon, glands, etc.	167	139	130	145	189	138	163	187	212	178	192	198	219
Loss in dissection	142	151	160	151	78	54	6	37	35	35	36	23	25
♂													
Mean													

APPENDIX IV (*continued*)

Composition of carcass at 200 lb.—high-protein series

		High-Low						Low-High						•	
		♂			♀			♂			♀				
		♂	♀	♂	♂	♀	♀	♂	♂	♀	♂	♀	♂		
Right shoulder g.	85	72	98	67	103	73	89	121	134	149	144	160	151		
Total weight	5110	5380	5590	5353	5910	5880	5895	5170	5230	5200	4900	5600	5370	5290	
Bones:															
Scapula	147	142	155	148	174	176	175	148	121	200	207	215	234	160	
Humerus	242	252	271	255	283	281	282	214	139	144	142	156	156	228	
Radii-ulna	164	158	178	167	180	178	179	144	139	136	134	137	138	153-3	
Carpals	40	38	48	42	39	47	43	36	32	32	34	36	38	37	
Cannons	47	46	55	49	52	55	53-5	40	36	38	44	43	42	43	
Splints	12	14	17	14	15	18	16-5	12	10	11	13	12	12	12-3	
Dew claws (2)	11-3	10-3	13-0	11-5	12-2	11-7	12-0	9-9	10-5	10	10-2	9-5	9-7	9-7	
Pasterns (2)	21-0	20-5	24-6	22-0	33-3	33-3	23-2	17-2	16-6	17	19-3	19-7	18-5	19-2	
Coronets (2)	12-7	12-1	15-8	13-5	13-5	12-7	13-1	9-9	10-0	10	10-4	11-5	11-0	10-9	
Pedals (2)	6-7	6-4	10-6	7-9	8-2	7-3	7-8	6-1	5-3	5-7	6-2	6-8	6-5	6-5	
Naviculars and sesamoids (4)	5-8	5-6	5-8	5-7	5-9	4-9	5-4	4-6	4-9	4-7	4-2	3-9	3-9	4-0	
Total bones	710	705	794	736	806	815	810	642	585	613	664	670	691	675	
Muscle:															
Shoulder	2342	2434	2494	2423	2893	2880	2886	1873	1945	1909	2184	2547	2287	2337	
Arm	277	285	309	290	331	320	326	204	250	227	233	244	245	241	
Round cannon	11	16	19	15	19	21	20	9	11	10	13	15	16	17	
Total muscle	2630	2735	2822	2728	3243	3221	3232	2086	2206	2146	2430	2806	2548	2595	
Fat:															
Subcutaneous	978	1050	1142	1057	900	871	885	1522	1643	1582	986	1153	1133	1091	
Intermuscular (shoulder)	295	400	375	356	303	358	331	380	402	391	390	425	399	405	
Intermuscular (arm)	17	21	19	19	19	26	22	37	29	33	19	25	25	23	
Total fat	1290	1471	1536	1432	1222	1255	1238	1939	2074	2006	1395	1603	1557	1519	
Skin	224	233	240	232	338	327	333	243	228	235	216	273	323	270	
Tendon, glands, etc.	254	194	179	209	153	202	178	216	162	189	156	136	197	163	
Loss in dissection	2	22	19	14	148	60	104	44	+25	11	39	112	54	68	

APPENDIX IV (continued)

Composition of carcass at 200 lb.—high-protein series

	High-High						Low-Low						♀ Mean 16517	
	♂			♀			♂			♀				
	♂	♂ Mean	♂	♀	♀ Mean	♀	♂	♂ Mean	♂	♀	♀ Mean			
Thorax (g.)	83	70	106	84	74	99	93	107	71	80	82			
Total weight	17252	18251	17480	17661	18583	19413	18998	15852	16027	15948	15868			
Bones:														
Ribs and sternum	865	926	869	887	1009	1080	1044	914	964	970	950	1001	1073	
Vertebræ	648	639	684	664	772	711	742	690	700	765	718	702	811	
Total	1513	1585	1553	1551	1781	1791	1786	1604	1664	1735	1668	1703	1884	
Muscle	5500	5981	6103	5861	7055	6826	6940	7464	7081	7941	7495	7796	7647	
Fat:														
Subcutaneous	6439	5687	5626	5917	5503	6142	5822	3383	3677	3021	3361	3426	4004	
Intermuscular	2363	3534	2778	2892	2894	3359	3127	2334	2371	2148	2351	1755	2245	
Total	8802	9221	8404	8809	8397	9501	8949	5917	6048	5169	5712	5221	6249	
Skin	848	879	853	860	960	959	960	780	896	926	867	1005	1050	
Tendon, glands, etc.	46	15	39	33	39	31	35	35	36	28	33	28	30	
Loss in dissection	543	570	528	547	351	305	328	152	302	149	201	115	304	
													209	
Low-High														
	High-Low						Low-High							
	♂			♀			♂			♀				
Thorax (g.)	85	72	98	67	103	89	73	73	73	93	93	66	79	
Total weight	17107	17530	17863	17500	17838	16065	16952	19189	18671	18930	18162	17745	18040	
Bones:														
Ribs and sternum	859	916	949	908	1100	1059	1080	802	715	759	940	883	1010	
Vertebræ	624	650	724	666	749	708	728	564	516	540	628	628	623	
Total	1483	1566	1673	1574	1849	1767	1808	1366	1231	1299	1568	1502	1633	
Muscle	5807	6431	6891	6376	7652	7860	7756	5437	5268	5352	5928	6002	6201	
Fat:														
Subcutaneous	5822	6078	4892	5597	4528	3189	3859	7501	7556	7529	5758	5766	5699	
Intermuscular	3218	2182	3326	2909	2427	1959	2193	3687	3554	3620	3703	2972	3134	
Total	9040	8260	8218	8506	6955	5148	6952	11188	11110	11149	9461	8738	8708	
Skin	726	861	853	813	959	945	932	802	696	749	787	-855	974	
Tendon, glands, etc.	37	29	32	33	36	29	32	25	33	24	39	29	33	
Loss in dissection	14	383	196	198	387	316	362	371	343	357	379	619	493	
													497	

		High-High						Low-Low					
		\bar{x}											
Loin (g.)	83	70	106	Mean	84	74	Mean	99	71	107	Mean	80	82
Total weight (less psoas)	6465	6532	5349	6115	6110	6018	6064	5778	5932	5629	5780	5327	4892
Bones:													
Vertebrae	388	407	310	368	437	358	397	435	452	433	440	423	380
Number	6	6	5	—	6	5	—	6	6	6	—	6	5
Muscle:													
Loin	2033	2176	1979	2063	2253	2164	2209	2598	2371	2618	2596	2708	2301
Psoas	669	541	699	636	704	683	694	751	701	749	734	814	731
Fat:													
Subcutaneous	3125	2791	2137	2684	2422	2528	2475	1592	1855	1619	1689	1371	1399
Intermuscular	460	465	439	455	427	537	482	577	511	453	513	305	354
Total	3585	3256	2576	3139	2849	3065	2957	2169	2366	2072	2202	1676	1753
Skin	512	481	377	456	419	413	416	438	492	467	466	467	408
Tendon, glands, etc.	30	32	16	26	30	14	22	22	20	21	21	20	22
Loss in dissection	+83	181	91	63	122	4	63	116	31	18	55	33	28
		High-Low						Low-High					
Loin (g.)	85	72	98	Mean	67	103	Mean	89	73	82	Mean	93	66
Total weight (less psoas)	7749	5517	6222	6496	6335	6014	6175	8243	6938	7591	6520	6897	6452
Bones:													
Vertebrae	390	355	438	394	459	457	458	324	287	306	397	363	386
Number	6	6	6	—	6	6	—	6	6	—	6	6	—
Muscle:													
Loin	2409	2148	2415	2324	2760	2776	2768	2228	1976	2102	2204	2420	2138
Psoas	677	696	693	689	757	896	827	646	545	596	651	669	650
Fat:													
Subcutaneous	3596	1975	2197	2589	1960	1638	1799	4312	3638	3975	2725	2874	2562
Intermuscular	730	593	688	670	520	405	463	674	448	561	628	621	657
Total	4326	2568	2885	3259	2480	2043	2262	4986	4086	4536	3353	3495	3219
Skin	474	358	451	428	492	558	525	485	408	447	448	487	473
Tendon, glands, etc.	22	20	20	21	17	19	18	15	13	14	19	18	18
Loss in dissection	128	68	13	70	127	161	144	205	168	186	99	114	207
		High-Low						Low-High					
Loin (g.)	83	70	106	Mean	84	74	Mean	99	71	107	Mean	80	82
Total weight (less psoas)	6465	6532	5349	6115	6110	6018	6064	5778	5932	5629	5780	5327	4892
Bones:													
Vertebrae	388	407	310	368	437	358	397	435	452	433	440	423	380
Number	6	6	5	—	6	5	—	6	6	6	—	6	5
Muscle:													
Loin	2033	2176	1979	2063	2253	2164	2209	2598	2371	2618	2596	2708	2301
Psoas	669	541	699	636	704	683	694	751	701	749	734	814	731
Fat:													
Subcutaneous	3125	2791	2137	2684	2422	2528	2475	1592	1855	1619	1689	1371	1399
Intermuscular	460	465	439	455	427	537	482	577	511	453	513	305	354
Total	3585	3256	2576	3139	2849	3065	2957	2169	2366	2072	2202	1676	1753
Skin	512	481	377	456	419	413	416	438	492	467	466	467	437
Tendon, glands, etc.	30	32	16	26	30	14	22	22	20	21	21	20	22
Loss in dissection	+83	181	91	63	122	4	63	116	31	18	55	33	28

APPENDIX IV (continued)

Composition of carcass at 200 lb.—high-protein series

High-High										Low-Low									
High-High					Low-Low					High-High					Low-Low				
	\bar{x}	\bar{x}	\bar{x}	\bar{x}		\bar{x}	\bar{x}	\bar{x}	\bar{x}		\bar{x}	\bar{x}	\bar{x}	\bar{x}		\bar{x}	\bar{x}	\bar{x}	\bar{x}
Pelvis (g.)	83	70	106	Mean	\bar{x}	84	74	Mean	\bar{x}	71	71	71	71	Mean	\bar{x}	80	82	82	Mean
Total weight	5953	6426	5936	6105	Mean	5842	6281	6062	5771	5591	5136	5499	6222	5384	5785				
Bones:																			
Pelvis	375	415	402	397	449	445	447	466	432	441	446	465	512	488					
Sacral vertebrae	133	122	117	124	126	159	143	155	142	141	146	148	181	164					
Caudal vertebrae	22	20	33	25	29	24	26	16	19	24	20	25	28	27					
Total	530	557	552	546	604	628	616	637	593	606	612	638	721	679					
Muscle	1848	2117	1957	1974	2108	2106	2107	2434	2264	2127	2275	2857	2214	2536					
Fat:																			
Subcutaneous	2542	2869	2484	2632	2267	2548	2408	1883	1923	1681	1829	1783	1544	1664					
Intermuscular	204	342	333	323	286	482	384	340	301	271	304	357	359	358					
Total	2836	3211	2817	2955	2553	3030	2792	2223	2224	1952	2133	2140	1903	2022					
Skin	311	340	287	313	317	300	309	305	360	290	318	440	356	398					
Tendon, glands, etc.	169	90	99	119	75	101	88	103	89	115	102	93	97	95					
Loss in dissection	259	111	224	198	185	116	150	69	61	46	59	54	57	55					
High-Low										Low-High									
	\bar{x}	\bar{x}	\bar{x}	\bar{x}		\bar{x}	\bar{x}	\bar{x}	\bar{x}		\bar{x}	\bar{x}	\bar{x}	\bar{x}		\bar{x}	\bar{x}	\bar{x}	\bar{x}
Pelvis (g.)	85	72	98	Mean	\bar{x}	67	103	Mean	\bar{x}	89	343	347	345	385	380	401	389		
Total weight	6018	5120	5070	5403	Mean	6384	5733	6059	5837	5796	5816	5671	5878	6019	5856				
Bones:																			
Pelvis	408	415	442	422	482	438	460	146	111	89	100	104	119	123	115				
Sacral vertebrae	131	116	147	131	163	129	146	111	19	19	19	22	29	21	24				
Caudal vertebrae	20	16	24	20	30	26	28	28	19	19	19	22	29	21	24				
Total	559	547	613	573	676	593	634	477	451	464	511	528	545	528					
Muscle	1913	1723	1773	1803	2541	2372	2457	1560	1613	1586	1830	1962	1841	1878					
Fat:																			
Subcutaneous	2640	1972	1868	2160	2233	1693	1964	2824	2881	2853	2493	2515	2648	2552					
Intermuscular	340	343	322	335	301	375	338	517	397	457	329	343	355	342					
Total	2980	2315	2190	2495	2534	2070	2302	3341	3278	3310	2822	2958	3003	2894					
Skin	282	281	290	284	375	456	416	251	255	253	335	290	391	339					
Tendon, glands, etc.	94	117	100	104	123	112	117	94	97	95	94	79	104	92					
Loss in dissection	190	137	104	144	136	130	133	114	102	108	79	79	161	135	125				

	Left leg (g.)	High-High						Low-Low					
		♂			♀			♂			♀		
		♂	♂ Mean	♀	♀ Mean	♂	♂ Mean	♀	♀ Mean	♂	♂ Mean	♀	♀ Mean
Total weight *	6250	6140	6030	6140	6570	6390	6480	6846	6945	6657	6816	6680	6621
Bones:													
Femur	250	264	255	256	263	264	263.5	291	301	293	295	290	295
Tibia-fibula	187	192	190	193	193	193	193	221	210	214	215	211	210
Patella	14	16	15	15	16	15	15.5	16	16	18	17	16	15
Calcaneum	39	37	38	39	36	37	37.5	43	42	39	41	39	37
Astragalus	36	37	35	36	32	30	31	35	38	33	35	32	29
Tarsals	28	29	29	29	28	25	26.5	31	32	30	31	28	28
Cannons	51	53	56	51	52	51	51.5	57	59	57	52	52	50
Splints	12	15	13	13	13	13	13	14	15	14	14	15	13
Dew claws (2)	11.0	10.3	9.5	10.3	10.4	9.0	9.7	11.1	10.6	10.7	10.8	10.9	10.4
Pasterns (2)	21.8	20.7	21.7	21.4	21.2	19.6	20.4	25.0	23.7	22.6	23.8	22.6	22.4
Coronets (2)	12.9	12.5	12.4	12.6	12.6	11.5	12.1	15.1	14.4	13.3	14.3	13.0	13.1
Pedals (2)	7.4	6.0	7.1	6.8	6.8	5.8	5.8	8.3	7.0	7.8	7.7	7.5	7.4
Naviculars and sesamoids (4)	4.3	4.1	4.6	4.3	4.1	4.2	4.2	5.8	4.4	5.2	5.1	4.7	4.8
Total bones	674	699	687	686	689	678	684	773	773	756	767	742	734
Muscle:													
Thigh	2643	2603	2577	2608	3054	2811	2932	3420	3343	3202	3322	3440	3188
Leg	480	494	525	500	546	512	529	608	596	582	599	629	513
Round cannon	18	13	17	16	20	19	20	28	28	31	29	23	22
Total muscle	3141	3110	3119	3124	3620	3342	3481	4056	3967	3825	3950	4092	3723
Fat:													
Subcutaneous	1495	1466	1289	1417	1346	1449	1397	1115	1219	1069	1134	875	1115
Intermuscular	211	189	225	208	186	196	191	222	221	278	240	201	218
(thigh)													
Intermuscular	54	41	55	50	38	48	43	40	33	33	36	48	47
(leg)													
Total fat	1760	1696	1569	1675	1570	1693	1631	1377	1473	1380	1410	1124	1380
Skin	339	336	308	328	394	314	354	349	402	407	386	388	420
Tendon, glands, etc.	223	254	216	231	207	239	223	247	279	249	258	256	300
Loss in dissection	113	45	131	96	90	124	107	44	51	40	45	78	64
													71

(39)

APPENDIX IV (continued)

Composition of carcass at 200 lb.—high-protein series

	High-High						Low-Low					
	\bar{x}											
Right leg (g.)	83	70	106	84	74	99	71	107	80	82	82	Mean
Total weight	6000	6020	6190	6070	6390	6350	6370	6820	7080	6890	6600	6509
Bones:												6554
Femur	249	276	253	259.3	263	267	265	294	297	298	286	297
Tibia-fibula	185	201	193	193	180	191	190.5	217	213	217	216	216
Patella	14	17	15	15.3	17	16	16	16	16	18	17	16
Calcaneum	40	42	38	40	38	37	37.5	43	41	39	41	39
Astragalus	36	38	34	36	32	31	31.5	35	39	33	36	31
Tarsals	27	31	29	29	27	28	28	30	32	30	31	30
Cannons	51	55	57	64.3	52	53	52	57	59	55	57	51
Splints	13	15	15	14.3	13	14	13.5	14	15	15	15	14
Dew claws (2)	9.9	10.9	10.3	10.4	9.2	9.8	10.4	11.8	10.7	11.4	10.0	10.0
Pasterns (2)	21.2	21.4	22.6	21.7	21.2	21.8	21.5	23.7	24.3	22.8	23.6	22.5
Coronets (2)	12.9	13.2	13.1	13.1	12.6	12.4	12.5	13.6	14.6	13.4	13.9	13.1
Pedals (2)	6.9	7.2	6.8	5.7	7.0	6.8	8.3	7.0	7.4	7.6	7.8	7.8
Naviculars and sesamoids (4)	3.9	4.4	4.7	4.3	4.2	4.3	4.2	4.8	4.9	5.0	4.8	5.1
Total bones	670	731	692	698	688	691	689	767	775	765	750	751
Muscle:												740
Thigh	261.4	2482	2640	2579	3013	2747	2880	3335	3439	3425	3400	3435
Leg	480	493	537	503	556	505	520	619	606	601	609	603
Round cannon	15	13	19	16	21	15	18	26	26	29	27	26
Total muscle	3109	2988	3196	3098	3590	3267	3418	3980	4071	4055	4036	4064
Fat:												3936
Subcutaneous	1382	1374	1405	1387	1211	1410	1311	1149	1254	1069	1157	926
Intermuscular (thigh)	193	218	207	206	190	222	206	236	225	187	223	159
Intermuscular (leg)	6	58	51	57	51	53	52	43	34	35	37	41
Total fat	1636	1650	1663	1650	1452	1685	1569	1448	1513	1291	1417	1127
Skin	297	308	338	314	324	339	332	311	368	369	349	365
Tendon, glands, etc.	222	211	226	219	231	195	213	273	307	259	279	254
Loss in dissection	66	132	75	91	105	173	139	41	46	31	39	61
												45

		High-Low				Low-High			
		♂		♀		♂		♀	
		♂	♂	♀	♀	♂	♂	♀	♀
Left leg (g.)		85	72	98	67	103	73	66	79
Total weight		6520	6600	7430	6850	7640	7190	6010	6310
Bones:									
Femur	268	248	287	268	286	295	290	208	238
Tibia-fibula	187	183	219	196	213	224	218-5	165	174
Patella.	15	15	17	16	16	19	18	12	14
Calcaneum	39	34	44	39	39	43	41	34	32
Astragalus	35	31	40	35	34	37	36	29	29
Tarsals	30	26	33	30	38	30	29	24	28
Cannons	52	50	63	55	57	58	58	45	50
Splints	12	11	14	12	14	14	14	12	10
Dew claws (2)	9-1	9-5	11-7	10-1	10-1	10-1	10-0	8-0	7-1
Pasterns (2)	21-5	21-2	26-0	22-9	23-9	24-4	24-2	18-4	18-1
Coronets (2)	12-4	12-5	16-6	13-5	13-1	13-5	13-5	10-6	10-8
Patellae (2)	6-4	7-4	8-7	7-5	7-4	6-8	7-1	5-5	5-8
Naviculars and sesamoids (4)	4-3	4-7	5-8	4-9	5-1	5-1	5-1	3-7	3-6
Total bones	692	653	785	710	747	780	764	590	559
Muscle:									
Thigh	2936	2955	3154	3015	3715	3650	3683	2427	2399
Leg	535	558	577	557	658	638	648	448	453
Round cannon	22	25	24	24	26	26	26	20	20
Total muscle	3493	3538	3755	3596	4399	4314	4357	2895	2846
Fat:									
Subcutaneous	1504	1491	1865	1620	1529	1123	1326	1696	1816
Intermuscular (thigh)	192	233	286	237	189	211	200	213	263
Intermuscular (leg)	43	43	45	44	38	38	38	61	48
Total fat	1739	1767	2196	1901	1756	1372	1564	1970	2127
Skin	312	361	379	351	422	379	400	286	282
Tendon, glands, etc.	251	243	279	257	273	291	282	223	221
Loss in dissection	33	38	36	35	43	54	48	46	65

APPENDIX IV (*continued*)

Composition of carcass at 200 lb.—high-protein series

Organs and offals at 200 lb.—high-protein series

Organ and offals	High-High						Low-Low					
	\bar{x}											
g.	83	70	106	84	74	99	71	107	80	82	80	82
Empty live weight	80960	82429	81402	81597	82105	84861	81118	82563	80006	81229	81332	82608
Skin and hair	520	505	585	537	510	529	520	945	1018	918	961	1147
Hoofs: Fore	47	41	42	43	46	48	47	42	48	45	45	44
Hind	32	28	31	30	31	30	31	33	38	34	35	33
Blood (total)	4184	4126	3938	4083	4016	4562	4289	3655	3756	3730	3819	3970
Glands (total)	357	429	352	379	393	381	387	296	347	229	291	303
Diaphragm	397	409	436	414	420	388	404	488	440	537	488	483
Heart	307	265	276	283	262	314	288	314	269	298	294	472
Pericardium and blood vessels	179	213	181	191	208	295	251	365	267	360	327	319
Lungs and trachea	634	656	606	632	684	727	706	583	657	688	643	682
Thoracic organs	1517	1543	1499	1520	1574	1724	1649	1750	1623	1883	1752	1772
Oesophagus	58	53	46	52	57	50	54	84	97	85	85	97
Stomach	615	696	500	604	536	530	533	643	681	707	677	754
Small intestine	1777	1479	1720	1659	1635	1459	1547	1159	1205	1441	1269	1307
Caecum	136	139	164	146	126	146	136	109	150	119	126	134
Large intestine	774	727	830	777	812	773	792	700	803	706	737	825
Rectum	192	253	226	224	244	169	206	205	221	226	217	216
Total tract	3552	3347	3486	3462	3410	3127	3268	2900	3150	3284	3111	3306
Caul	98	134	154	129	120	162	141	143	125	127	132	116
Mesentery	1006	1045	1174	1075	993	1344	1168	1000	807	869	1039	1235
Liver	2634	2277	2650	2520	2327	2386	2357	1837	1627	1808	1757	1816
Gall bladder	31	43	29	34	37	78	57	30	76	28	45	22
Spine	82	100	67	83	84	99	92	65	81	84	90	91
Pancreas	119	121	116	119	102	116	109	102	100	86	96	93
Kidneys	490	374	410	425	413	377	377	259	226	242	226	346
Kidney and kidney fat	1225	1524	1784	1511	1143	1629	1386	1551	1163	1275	1330	1185
Bladder	49	52	57	52	57	51	44	62	70	58	55	50
Total abdominal	9286	9017	9827	9410	8613	9399	9006	7931	7426	7795	7717	7677
Uterus and vagina	—	—	—	—	219	187	203	—	—	—	333	242
Penis and testiculae seminariales	96	79	82	86	—	—	—	69	78	63	70	—
Total offals	16039	15768	16456	16088	15402	16860	16131	14721	14358	14723	14601	15129
<i>Length of alimentary tract (cm.) at 200 lb.—high-protein series</i>												
Small intestine	2240	2140	2192	2191	2168	2174	2171	2108	2170	1920	2066	1893
Caecum	23	24	20	22	26	25	21	24	21	19	21	23
Large intestine	382	336	326	348	360	410	385	349	469	314	377	461
Rectum	117	141	114	124	136	107	121	109	125	127	104	121
Total length	2762	2641	2652	2685	2690	2716	2703	2587	2738	2380	2568	2423
												2659
												2541

APPENDIX IV (continued)

Organs and offals at 200 lb.—high-protein series

Organs and offals	High-Low						Low-High					
	♂			♀			♂			♀		
	♂	♂	♂	♀	♀	♀	♂	♂	♂	♀	♀	♀
g.	86	72	98	67	103	89	73	93	93	66	79	79
Empty live weight	85780	81535	84730	84015	82208	85079	87162	87049	82652	8776	84534	84821
Skin and hair	647	681	753	694	628	1015	527	639	583	586	417	630
Hoofs: Fore	38	39	40	44	52	48	38	37	39	36	40	38
Hoofs: Hind	28	38	34	30	35	34	35	34	33	33	28	30
Blood (total)	4526	3519	3485	3843	3933	4099	4016	4331	3866	3998.5	4323	4012
Glands (total)	310	353	248	303	267	287	359	373	366	276	337	303
Diaphragm	473	470	440	461	434	465	449.5	375	352	441	495	448
Heart	287	284	286	285.7	268	249	258.5	297	249	273	272	267
Pericardium and blood vessels	220	199	381	266.7	244	270	257	373	278	325.5	335	265
Lungs and trachea	542	665	628	612.3	951	705	828	578	594	586	518	593
Thoracic organs	1524	1618	1735	1626	1897	1689	1793	1623	1473	1548	1572	1626
Oesophagus	64	61	64	63	57	75	66	55	47	51	55	62
Stomach	639	588	534	587	613	591	602	624	499	561.5	501	492
Small intestine	1410	1319	1059	1263	1302	1113	1208	1600	1442.5	1381	1366	1406
Caecum	139	127	160	112	135	156	145.5	126	150	138	137	160
Large intestine	696	674	680	683.3	933	751	842	720	747	733.5	696	720
Rectum	246	178	205	209.7	216	255	235.5	191	193	192	223	156
Total tract	3194	2947	2702	2948	3256	2941	3099	3316	2921	3118.5	2905	2888
Caul	123	128	160	137	129	119	124	135	280	197.5	166	159
Mesentery	977	983	1313	1091	1104	855	979	1190	1074.5	1333	1362	1325
Liver	2240	1680	1710	1877	1695	1650	1650	2625	2353	2489	1902	1943
Gall bladder	21	28	19.3	51	15	33	49	12	30.5	1.4	81	45
Spleen	80	86	78	81.3	86	76	81	70	64	67	92	91
Pancreas	115	89	97	100.3	97	89	93	163	112	137.5	113	108
Kidneys	429	231	285	315	245	263	249	438	357	397.5	314	306
Leaf and kidney fat	1599	1651	1581	1610	1216	1117	1190	1904	2205	2054.5	1790	1984
Bladder	45	53	40	46	53	66	59	46	46	46	67	52
Total abdominal	8823	7837	7994	8225	7978	7136	7557	9705	9320	9612	867.5	8981
Uterus and vagina or	—	—	—	—	266	205	235.5	—	—	—	184	202
Penis and vesiculae seminales	144	66	66	92	—	—	102	116	109	—	—	—
Total offals	16040	14161	14355	14852	15048	14517	14783	16720	15847	14682	16285	16080
Length of alimentary tract (cm.) at 200 lb.—high-protein series												
Small intestine	2056	2204	2021	2094	2107	2055	2081	2287	2083	2185	2022	2233
Caecum	24	28	24	25	24	26	25	20	26	23	20	21
Large intestine	334	386	386	369	353	347	356	398	389	393	265	344
Rectum	123	127	121	124	121	91	142	116	129	123	138	127
Total length	2537	2745	2552	2612	2575	2570	2572	2821	2627	2724	2435	2778

Carcass measurements at 200 lb.—high-protein series

Carcass measurements mm.	High-High						Low-Low					
	δ	δ^2	δ^3	δ^4	δ^5	δ^6	δ^7	δ^8	δ^9	$\delta^{\frac{1}{2}}$	$\delta^{\frac{1}{3}}$	$\delta^{\frac{1}{4}}$
	70	106	Mean	84	74	74	71	99	71	107	80	82
Body length:												
Head	34	44	32	37	41	41	47	47	42	44	40	45
Neck	151	159	162	157	136	156	149	174	186	170	149	176
Thorax	418	477	542	479	482	442	450	427	439	434	451	442
Loin	206	213	182	200	224	204	214	250	242	245	227	205
Pelvis	212	180	211	198	212	205	204	175	195	191	213	216
Total body length	1045	1082	1034	1054	1078	1081	1080	1099	1113	1092	1073	1108
Carcass:												
Length of side	750	758	731	746	760	795	778	765	765	760	777	769
Length of leg	548	570	565	561	578	574	650	637	610	632	618	620
Depth of rib	345	350	348	351	349	350	346	348	351	348	353	357
Forearm length	212	213	210	212	220	223	222	245	234	238	228	227
Forearm circumference	238	232	239	233	230	233	233	231	229	232	233	231
Circumference base of tail	86	78	82	95	82	95	89	85	86	106	92	96
Foretroster	92	95	93	93	95	98	96	107	102	105	97	97
Streak thickness:												
Breast	27	27	26	27	31	32	32	30	26	29	26	28
Middle	25	25	28	26	29	29	29	28	24	29	27	30
Inguinal	25	25	25	25	31	29	29	28	24	29	29	29
Back fat thickness:												
(a) Shoulder: Inner Outer	36	32	35	34	28	34	31	24	27	30	27	30
Total	9	10	7	9	9	7	8	8	8	10	9	9
(b) Loin: Inner Outer	45	42	42	43	37	41	39	32	36	38	35	39
Total	16	16	15	17	14	16	15	9	8	7	8	8
(c) Rump: (1) (2) (3)	9	7	7	9	9	9	5	8	5	6	6	5
Mean rump	38	34	32	35	31	34	32	18	23	16	19	18
Mean back fat	36	33	32	34	31	33	32	21	25	22	23	22
Cut at last rib:												
A	74	79	71	75	84	79	81	83	84	92	86	95
B	36	37	36	36	39	37	38	45	39	36	40	39
C	27	27	23	26	22	23	22	12	16	11	13	9
H	42	40	34	39	33	34	33	21	22	21	16	17
Eye-shape index	486	507	487	464	468	466	452	464	391	466	500	337
Thickness of skin	3	3	3	3	3	3	3	3	3	3	3	3
Colour of muscle:												
Last rib	2 P	3 P	2 P	2 P	3 P	4 P	3 P	10 B	10 B	10 B	10 B	10 B
Diaphragm	10 B	6 P	6 P	—	10 B	11 B	10 B	11 B	12 B	11 B	11 B	11 B

(45)

P = pork scale; B = beef scale.

Carcass measurements at 200 lb.—high-protein series

Carcass measurements mm.	High			Low			High			Low			High			Low		
	$\frac{1}{2}$	$\frac{2}{3}$	Mean															
Body length:																		
Head	36	33	46	38	37	42	39	37	31	34	45	40	48	48	48	44	44	44
Neck	177	185	206	189	182	187	184	180	167	141	162	168	168	168	168	157	157	157
Thorax	420	438	434	438	484	436	460	410	425	417	428	425	449	449	449	434	434	434
Loin	234	239	233	235	245	242	243	236	217	226	244	227	236	236	236	236	236	236
Pelvis	202	175	201	193	220	212	205	212	186	193	210	210	207	207	207	199	199	199
Total body length	1068	1081	1120	1090	1112	1101	1106	1020	1051	1036	1078	1035	1089	1067	1067	1067	1067	1067
Carcass:																		
Length of side	780	783	793	785	805	785	795	750	712	731	782	775	757	757	757	765	765	765
Length of leg	569	595	643	602	608	610	609	550	563	566	577	575	570	574	574	574	574	574
Depth of rib	358	348	334	356	351	350	358	354	354	334	334	334	353	353	353	348	348	348
Forearm length	225	233	230	229	220	234	227	192	192	199	207	199	222	215	215	215	215	215
Forearm circumference	229	220	242	230	241	228	234	221	227	224	230	230	225	228	228	228	228	228
Circumference base of tail	83	92	89	88	92	99	92	95	90	85	87	80	84	84	84	83	83	83
Fore trotter	102	98	110	103	109	108	108	89	83	86	89	97	90	93	93	93	93	93
Streak thickness:																		
Breast	30	29	40	33	33	29	31	26	24	26	32	30	31	31	31	31	31	31
Middle	28	34	42	35	35	29	32	30	23	27	32	30	33	33	33	32	32	32
Inguinal	40	28	31	33	33	25	29	45	21	33	33	26	31	31	30	30	30	30
Back fat thickness:																		
(a) Shoulder: Inner	34	29	33	32	26	27	27	45	44	45	45	35	39	39	39	35	35	35
Outer	10	8	7	8	10	9	9	10	13	11	11	9	10	10	10	10	10	10
Total	44	37	40	40	36	36	36	55	57	56	46	48	41	45	45	45	45	45
(b) Loin: Inner	13	11	11	12	11	7	9	20	27	24	16	19	14	16	16	16	16	16
Outer	9	7	7	8	6	5	6	13	13	13	8	11	8	9	9	9	9	9
Total	22	18	18	20	17	12	14	33	40	37	24	30	22	25	25	25	25	25
(c) Rump: (1)	39	28	26	31	28	19	24	45	45	45	45	39	31	32	32	32	32	32
(2)	31	20	21	24	19	13	16	38	40	40	22	28	27	27	27	27	27	27
(3)	37	30	31	33	27	21	24	53	44	49	31	36	34	34	34	34	34	34
Mean rump	36	26	26	29	25	18	21	46	42	44	26	35	31	31	31	31	31	31
Mean back fat	34	27	28	30	26	22	24	45	46	46	32	38	32	34	34	34	34	34
Cut at last rib:																		
A	77	84	82	81	91	90	91	68	71	70	78	75	78	77	77	77	77	77
B	41	32	34	36	43	45	44	40	29	34	35	41	41	39	39	39	39	39
C	28	22	19	23	15	11	13	35	39	37	22	25	23	24	24	24	24	24
H	38	26	27	30	23	16	19	51	49	50	32	36	34	34	34	34	34	34
Eye-shape index	532	381	415	443	472	500	486	588	408	498	449	547	526	507	507	507	507	507
Thickness of skin	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Colour of muscle:																		
Last rib	2 P	5 P	4 P	3 P	5 P	4 P	2 P	3 P	3 P	4 P	4 P	4 P	4 P	4 P	4 P	4 P	4 P	4 P
Diaphragm	10 B	11 B	11 B	11 B	10 B	10 B	11 B	10 B	10 B	10 B	10 B	10 B	10 B	10 B	10 B	10 B	10 B	10 B

(46)

P = pork scale; B = beef scale.

Weekly live weights—200 lb. high-protein series (birth—200 lb.)

Fig. no.	Age in weeks																							
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
H.H.	70	6	12	17	23	29	34	40	44	53	64	75	85	100	109	120	130	142	152	162	173	185	200	200
H.L.	72	5	9	11	13	15	19	22	28	36	43	55	64	70	75	86	94	102	110	120	139	151	160	160
L.H.	73	5	8	10	12	14	16	17	19	20	22	25	28	30	34	37	42	52	64	73	82	89	100	116
L.L.	71	6	10	13	17	21	25	29	31	33	34	37	36	38	41	44	46	50	54	59	65	70	74	80
H.H.	83	6	11	13	15	18	20	24	29	37	44	54	61	70	78	88	97	104	116	127	149	159	171	188
H.L.	85	6	10	12	14	19	22	26	32	38	47	57	62	70	81	88	94	100	110	118	131	138	146	162
L.H.	89	6	9	10	12	14	17	19	19	21	24	27	28	32	36	38	40	52	64	80	94	107	118	138
L.L.	99	7	9	11	10	15	18	22	26	29	30	35	37	37	39	41	45	50	56	60	66	72	79	84
H.H.	106	6	11	15	22	27	32	39	42	48	61	73	83	92	100	112	124	134	145	159	174	185	200	200
H.L.	98	6	9	11	14	18	21	26	31	40	47	54	62	70	77	85	92	100	110	120	131	142	149	155
L.H.	93	6	7	8	10	13	16	19	22	24	31	34	36	37	38	41	49	60	69	75	82	90	101	109
L.L.	107	7	10	11	15	16	18	21	22	26	28	30	33	35	38	41	45	52	58	61	66	71	75	80
H.H.	74	4	9	12	16	19	22	24	27	33	38	47	55	63	72	84	93	100	106	110	122	133	148	157
H.L.	103	6	10	13	17	19	22	28	35	40	52	62	73	83	92	101	106	109	119	132	136	147	156	163
L.H.	79	4	8	10	13	15	16	19	22	26	29	33	36	42	47	49	55	65	74	83	92	102	114	121
L.L.	84	4	6	8	10	13	16	17	18	21	25	28	31	34	38	41	46	50	56	61	68	74	80	80
H.H.	66	6	11	13	16	21	26	33	38	46	55	62	68	77	87	100	107	115	126	132	147	155	171	188
H.L.	67	6	11	15	19	23	28	34	50	58	68	75	92	106	116	124	132	140	147	154	160	168	177	182
L.H.	66	7	11	14	17	21	25	30	31	32	37	38	40	43	46	48	53	68	71	86	99	107	122	134
L.L.	80	5	8	10	13	15	16	19	22	23	25	29	33	37	42	47	50	52	56	60	63	69	75	80

(47)

Fig. no.	Age in weeks																								
	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	
H.H.	70	166	175	183	188	194	200																		
H.L.	72	140	157	169	178	189	197																		
L.H.	73	100	102	108	113	120	124	128	133	140	144	149	155	160	166	172	177	182	188	193	197	199			
H.H.	83	170	180	190	200																				
H.L.	85	168	180	192	200																				
L.H.	99	100	105	108	114	120	125	129	133	139	144	152	160	164	170	172	182	188	192	195	198				
H.H.	106	169	176	180	184	187	190	194	196	200															
H.L.	93	119	130	140	150	160	172	182	194	200															
L.H.	107	90	96	101	104	107	109	113	118	122	127	130	134	139	148	153	160	166	170	175	180	185	194	198	
H.H.	74	192	200																						
H.L.	103	178	182	185	190	194	196	199																	
L.H.	79	132	142	153	163	174	187	197																	
H.H.	84	185	188	193	197	200																			
H.L.	66	150	160	174	190	200																			
L.H.	80	89	92	97	102	108	112	116	120	124	128	131	136	144	147	153	159	164	170	175	180	185	192	197	

APPENDIX IV (*continued*)*Food consumption and utilization from weaning to 16 weeks*

Pig no.	Separated milk gal.	Meal up to 16 weeks lb.	Live weight 8 weeks lb.	Weight 16 weeks lb.	Gain lb.	Meal per lb. gain	Milk per lb. gain	Meal equivalent per lb. gain
High Plane								
69	56	304	40	113	73	4.16	0.77	4.93
95	56	214	29	87	58	3.69	0.96	4.65
101	56	296	33	100	67	4.42	0.83	5.25
70	56	304	44	130	86	3.53	0.65	4.18
106	56	296	42	124	82	3.61	0.68	4.29
83	56	232	29	97	68	3.41	0.82	4.23
84	56	232	38	107	69	3.36	0.81	4.17
74	56	190	27	93	66	2.88	0.85	3.73
67	56	304	44	124	80	3.80	0.70	4.50
85	56	218	32	94	62	3.52	0.90	4.42
72	56	190	28	86	58	3.28	0.96	4.24
103	56	264	40	109	69	3.83	0.81	4.64
98	56	218	30	92	62	3.52	0.90	4.42
All pigs	728	3262	456	1356	900	3.62	0.81	4.43
Low plane								
68	28	64	26	42.5	16.5	3.88	1.70	5.58
92	28	60	16	31.5	15.5	3.87	1.81	5.68
100	28	61	25	47.0	22.0	2.77	1.27	4.04
66	28	64	30	48.0	18.0	3.56	1.56	5.12
89	28	86	19	40.0	21.0	4.10	1.33	5.43
73	28	68	19	42.0	23.0	2.96	1.22	4.18
79	28	67	24	44.0	20.0	3.35	1.40	4.75
93	28	86	22	41.0	19.0	4.53	1.47	6.00
71	28	64	31	48.0	17.0	3.76	1.65	5.41
82	28	67	16	38.0	22.0	3.05	1.27	4.32
80	28	74	22	49.0	27.0	2.74	1.04	3.78
99	28	68	26	45.0	19.0	3.58	1.47	5.05
107	28	62	22	45.0	23.0	2.70	1.22	3.92
All pigs	364	891	298	561	263	3.39	1.38	4.77

APPENDIX IV (continued)
Food consumption and utilization

	16 weeks—200 lb.						Weaning—200 lb.					
	Separated milk no.	Meal lb. gal.	Final weight lb.	Gain lb.	Meal per lb. gain	Milk per lb. gain	Total meal milk gal.	Total meal milk lb.	Total meal milk gal.	Milk per lb. gain	Meal equi- valent per lb.	Milk per lb. gain
High-High	70	44	335	206	76	4.41	0.58	4.99	100	63.9	162	3.94
	106	44	382	200	76	5.03	0.58	5.61	100	67.8	158	4.29
	83	59	515	200	103	5.00	0.57	5.57	115	74.7	171	4.37
	84	59	489	197	90	5.43	0.66	6.09	115	72.1	159	4.53
	74	69	617	198	105	5.88	0.65	6.53	125	80.7	171	4.72
All pigs	275	2338	1001	450	5.19	0.61	5.80	555	3592	821	4.38	0.67
High-Low	67	73	279	204	80	3.49	0.91	4.40	129	583	160	3.64
	85	69	326	200	106	3.07	0.65	3.72	125	544	168	3.24
	72	78	425	198	112	3.79	0.70	4.49	134	61.5	170	3.62
	103	82	315	198	89	3.54	0.93	4.47	138	57.9	158	3.66
	98	86	323	199	107	3.02	0.80	3.82	142	54.1	169	3.20
All pigs	388	1668	999	494	3.38	0.78	4.16	668	2862	825	3.47	0.81
Low-High	66	147	608	200	152	4.00	0.97	4.97	175	672	170	3.95
	89	138	577	200	160	3.60	0.86	4.46	166	663	181	3.66
	73	156	743	197	155	4.79	1.01	5.80	184	811	178	4.56
	79	163	811	197	153	5.30	1.07	6.37	191	878	173	5.07
	93	172	875	194	153	5.72	1.12	6.84	200	961	172	5.59
All pigs	776	3614	988	773	4.68	1.00	5.68	916	3985	874	4.56	1.05
Low-Low	71	231	528	197	149	3.54	1.55	5.09	259	592	166	3.57
	82	255	629	197	159	3.96	1.60	5.56	283	696	181	3.85
	80	250	580	197	148	3.92	1.69	5.61	278	664	175	3.74
	99	232	526	198	153	3.44	1.52	4.96	260	594	172	3.45
	107	237	553	198	153	3.61	1.55	5.16	265	615	176	3.49
All pigs	1205	2816	987	762	3.70	1.58	5.28	1345	3151	870	3.62	1.55

THE EFFECT OF A SUBMAINTENANCE DIET ON THE COMPOSITION OF THE PIG

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(With Plate 1 and Four Text-figures)

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INTRODUCTION

GROWTH in pigs has been shown by McMeekan (1940) to occur not by a simple proportionate increase in the body as a whole but rather by a series of waves starting from the head, the extremities of the limbs and from the tail and meeting in the loin. Furthermore, different parts of the body do not grow at the same rate at all times in the course of the development of the animal; of the three main tissues, bone reaches its maximum rate of growth first, then muscle and lastly fat. Pigs stunted in early life and fed well subsequently tend to have small bones, poorly developed muscle and an excessive proportion of fat, and conversely if the animal is fed well in early life and afterwards restricted it develops its bone and muscle and has a moderate covering of fat at the same body weight.

McMeekan (1940) has shown that the result of this is that at any given weight the composition of the animal's body is largely determined by the shape of its growth curve.

Hitherto, work on the composition of the pig's body has been largely confined to the effect of the plane of nutrition in determining the composition on a supermaintenance diet, and the present investigation was planned by McMeekan in order to obtain information as to the effect of

loss of weight on body composition and the order in which the events constituting this loss of weight occur. We required to know whether a pig could be made to grow backwards, i.e. whether, when the animal loses weight, the order of the tissues and the parts of the body from which the weight is lost is the reverse of that order in which weight is put on during growth.

Furthermore, it was hoped to throw some light on changes occurring in the tissues, particularly in the distribution and composition of the fat in the body of pigs losing weight.

It has been shown that the composition of the fat of the pig is, to a considerable extent, governed by its rate of growth. Thus if growth is slow, the fats in the food, usually unsaturated oils, are deposited as body fat, whereas if growth is rapid, fat is synthesized from carbohydrates and such fat is hard (Callow, 1935). Soft fats are objectionable in the carcass of bacon pigs as they lead to spoilage in the bacon and particularly in hams in which, owing to the long storage period, rancidity is liable to develop. It is desirable, therefore, to determine exactly the factors governing the deposition and mobilization of fat in the pig.

By chemical analysis of samples of fat from the carcasses of pigs after varying periods on a submaintenance diet it is possible to determine whether unsaturated fats are mobilized first or last or whether saturated and unsaturated fats are used indiscriminately.

MATERIALS AND METHODS

For this experiment five pigs, all castrated males from the same inbred strain of Large Whites used by McMeekan (1940), were available. Their ear numbers were 167, 173, 174, 175 and 177 and their pedigrees are given below:

Pedigrees of pigs showing extent of inbreeding

173	$\left\{ \begin{array}{l} 338 \\ 14 \end{array} \right. \begin{array}{l} \text{Foundation Boar} \\ \text{Foundation Sow} \end{array}$
174	$\left\{ \begin{array}{l} 338 \\ 344 \end{array} \right. \begin{array}{l} \\ \text{Foundation Sow} \end{array}$
175	$\left\{ \begin{array}{l} 338 \\ 344 \end{array} \right. \begin{array}{l} \\ \text{Foundation Sow} \end{array}$
167	$\left\{ \begin{array}{l} 338 \\ 15 \end{array} \right. \begin{array}{l} \\ \left\{ \begin{array}{l} 338 \\ 344 \end{array} \right. \begin{array}{l} \\ \end{array} \end{array}$
177	$\left\{ \begin{array}{l} 338 \\ 344 \end{array} \right. \begin{array}{l} \\ \end{array}$

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They were fed on a High Plane of nutrition so as to grow as quickly as possible to about 330 lb. The first to arrive at this weight (no. 175) was killed as a control, the actual live weight at slaughter being 327 lb. As the remaining pigs reached 327 lb. live weight they were removed to another pen and given an ample supply of bedding (wheat straw) and water but no other food. As a result of this submaintenance diet they lost weight and were killed at approximately equal weight intervals between 327 and 200 lb. The object of killing the last pig at 200 lb. was to obtain a carcass weight equal to that given by the High and Low Planes of nutrition castrated males used by McMeekan. The carcass composition of the two could then be compared.

It was thought that two difficulties might arise from keeping pigs on a submaintenance diet for a considerable period of time: (a) they might suffer from mineral and vitamin deficiencies; (b) they might develop a ketosis as a result of incomplete oxidation of the body fat. In order to counteract the first the pigs were turned out into a neighbouring paddock for short periods, when the weather permitted, in order to allow them to pick up green stuff and minerals. It was hoped that the pigs would eat enough straw to prevent them from suffering discomfort from hunger and derive sufficient carbohydrate from the straw for complete oxidation of the body fat. Actually the pigs did eat enough straw to prevent them from feeling hungry, and as they seemed to prefer partly fermented straw it is possible that they obtained enough carbohydrate in this way to prevent the development of a ketosis.

Tests were made periodically on the urine and samples of blood were sent to the Rowett Research Institute at Aberdeen where work on ketonaemia was in progress; no ketone bodies could be detected in either the blood or urine, and the sugar content of the blood was normal.

Throughout the experiment the pigs appeared to suffer no discomfort—they were quite as lively as normal pigs when disturbed and there was no clamouring for food.

The pigs were weighed twice weekly throughout the submaintenance period and on three successive days just before the required weight for killing was reached.

When this weight had been reached the animals were killed and completely dissected, using the technique described in detail by Hammond (1932), McMeekan (1940) and Pálsson (1939). The procedure involved in dissection is briefly as follows:

The pig is stunned with an electrolethaler, "stuck" and the blood collected and weighed. The body is then scalded and scraped and the

skin and hair weighed. The hoofs are removed and weighed. At this stage the body is photographed, lying on its side (Pl. 1, fig. 1) and weighed.

The animal is now eviscerated and the weight of each part is recorded. The alimentary tract is weighed full and empty (to determine the weight of its contents) and its length measured. The kidneys, leaf fat and psoas muscles are taken out and weighed, samples of the two latter being taken for chemical analysis. Samples are taken from leaf fat, mesenteric fat and psoas muscles for histological investigation. The dressed carcass is weighed and placed in cold storage at 0° C. for a short period of about 14 hr. After removal from cold storage the chilled carcass was photographed (Pl. 1, fig. 3), hanging from a gambrel of standard width, and measured.

The carcass is cut through at the level of the junction of the thorax and loin, and measurements of muscle and fat are taken and the cut section photographed (Pl. 1, fig. 2). The carcass is separated into anatomical joints, head, neck, thorax, loin, pelvis, legs and shoulders, and each part is weighed. The parts are then completely dissected into their constituents and the weight of each recorded.

During dissection samples of muscle and fat were taken from different parts of the carcass for histological and chemical work. Samples were passed to Dr E. H. Callow for determination of the chemical composition and to Prof. T. P. Hilditch for a determination of the different fatty acids present in the fat. The results of Prof. Hilditch's investigations have now been published (Hilditch & Pedley, 1939).

Since the same technique was used by practically the same operators who dissected McMeekan's pigs the various weights can be compared.

EFFECT ON LIVE WEIGHT, CARCASS WEIGHT AND CARCASS PERCENTAGE

Table 1 shows the effect of submaintenance on the live weight and carcass percentage of the pigs. The greatest loss in live weight was 67 % of the weight at the beginning of submaintenance after 135 days. At this stage the pig was certainly weak but not on the point of death.

Carcass percentage. There was a reduction of 2 % in the carcass percentage expressed in terms of empty live weight after 135 days' submaintenance, i.e. the carcass lost weight more rapidly than the offals, which is the converse of what happens during growth.

Losses. The losses on scalding and cooling remained approximately constant throughout at 10 and 4 lb. respectively. The loss on evisceration diminished in proportion to the live weight. Dissection losses were of

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the usual order of not more than 2 %, the greater losses being sustained in the heavier carcasses where the dissection time was longer.

Table 1. *Live weight, carcass weight, killing percentage and losses*

	Pig 175 control lb.	Pig 177 lb.	Pig 167 lb.	Pig 174 lb.	Pig 173 lb.
Weight at beginning of submain- tenance period	327	327	328	327	331
Weight at end of submain- tenance period	327	302	263	234	188
Weight after scalding	315	291	251	225	178
Loss	12	11	12	9	10
Hot carcass weight	258	240	208	181	145
Loss	57	51	43	44	33
Cold carcass weight	257	236.5	204	177	143
Loss	1	3.5	4	4	2
Empty live weight	310	286	248	217	177
Cold carcass weight as % of empty live weight at end of submaintenance period	82.9	82.7	82.3	81.6	80.8

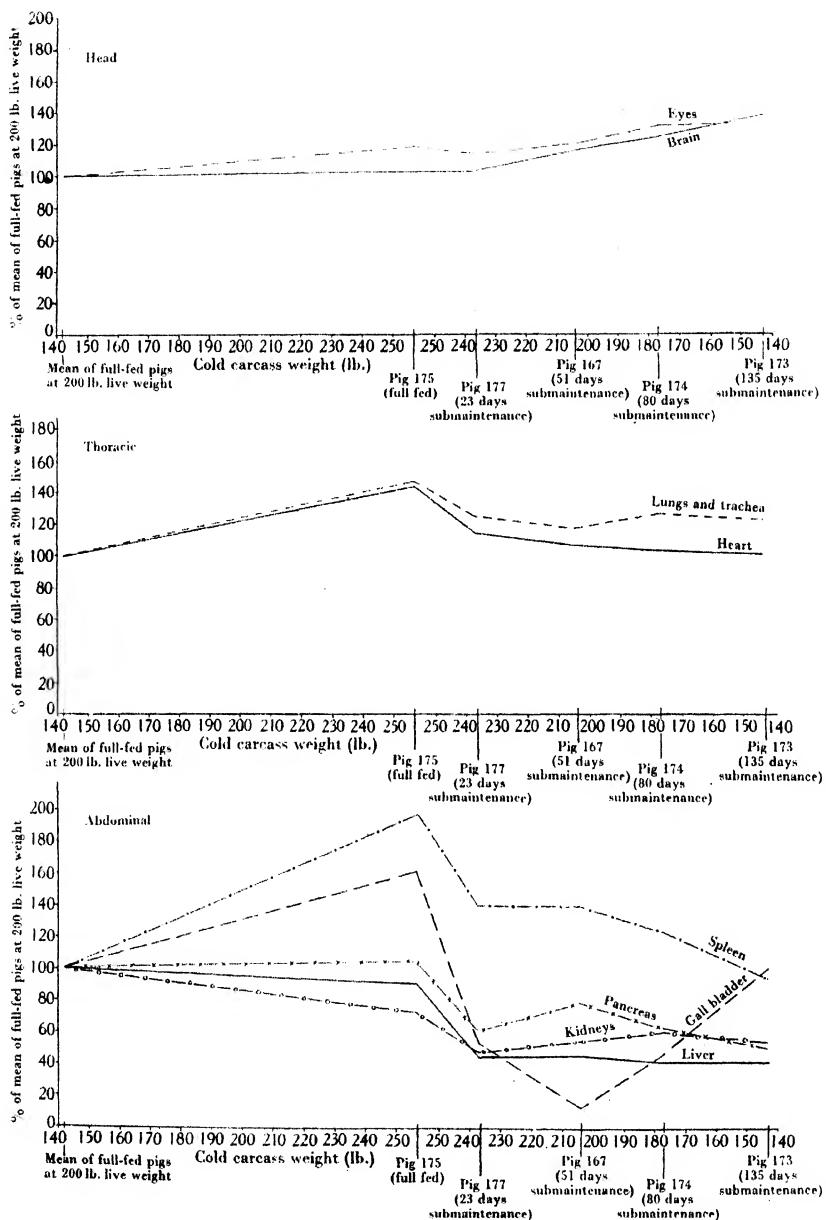
Body form. The general outline of the body changed (Pl. 1, fig. 1). From being deep in relation to leg length the body became shallow and more angular, these changes being the reverse of those occurring during growth (McMeekan, 1940). In particular, the relative length of leg, an early maturing part of the body was increased and there was a great reduction in the thickness of the loin, a late-maturing part of the body. This reduction is shown by the wrinkling of the skin in this region (Pl. 1, fig. 3).

EFFECT ON WEIGHT OF ORGANS (Text-fig. 1 and Table 2)

(1) *Head*

(a) *Brain.* The weight of brain in the submaintenance pigs increased progressively to 133.3 % of the control. The tendency for the brain to continue to grow during starvation has already been noted by Manassein (1869) in young rabbits and by Stewart (1918) in albino rats. Trowbridge *et al.* (1918, 1922) found that the brain weight in underfed steers at various ages was higher than in full-fed controls at the same body weight.

McMeekan's work with pigs (1940) showed that the growth of the brain, a very early developing organ, is a function of age and is independent of the level of nutrition on which the pig is placed after birth. In pigs fed on Low and High Planes of nutrition the weight of the brain is greater in the Low-Plane pigs at the same body weight; but if the



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Table 2. *Weight of organs*

	Pig 175 control wt. (g.)	Pig 177 wt. (g.)	Pig 167 wt. (g.)	Pig 174 wt. (g.)	Pig 167 wt. (g.)	Pig 173 wt. (g.)	Pig 173 control wt. (g.)	Pig 174 control wt. (g.)	Pig 173 control wt. (g.)
Blood	4877.0	4227.0	4825.0	3920.0	3746.0	86.7	98.9	80.4	76.8
Skin and hair	872.0	1225.0	960.0	1180.0	1023.0	140.5	110.1	135.3	117.3
Hoofs: Fore	61.0	61.0	69.0	70.0	60.0	100.0	113.0	114.8	98.4
Hind	47.0	44.0	54.0	49.0	47.0	93.6	114.9	104.3	100.0
Bladder	64.0	41.0	61.0	46.0	45.0	64.1	69.5	71.9	70.3
Neck thymus	46.0	94.0	98.0	14.0	17.0	204.3	213.0	30.4	37.0
Heart thymus	39.0	47.0	34.0	10.0	10.0	120.5	87.2	25.6	25.6
Gall bladder (full)	55.0	18.0	4.7	16.0	34.0	32.7	8.5	29.1	61.8
Diaphragm	546.0	542.0	483.0	583.0	396.0	99.3	88.0	106.8	72.5
Pancreas	125.0	73.0	93.0	75.0	60.0	58.4	74.4	60.0	48.0
Pericardium and blood vessels	506.0	468.0	277.0	478.0	288.0	92.5	54.7	94.5	56.9
Heart (without pericardium and blood vessels)	408.0	324.0	300.0	293.0	295.0	79.4	73.5	71.8	69.0
Lungs and trachea	935.0	790.0	744.0	799.0	773.0	84.5	79.6	85.5	82.7
Liver	2297.0	1112.0	1147.0	1055.0	1046.0	48.4	49.9	45.9	45.5
Spleen	163.0	116.0	116.0	102.0	78.0	71.2	71.2	62.6	47.9
Oesophagus	75.0	64.0	69.0	73.0	61.0	85.3	92.0	97.3	81.3
Stomach: Empty Contents	601.0	503.0	488.0	512.0	463.0	83.7	81.2	85.2	77.0
Small intestine: Empty Contents	3721.0	2926.0	2538.0	1395.0	78.6	68.6	68.5	68.5	68.5
Large intestine: Empty Contents	1366.0	828.0	833.0	864.0	777.0	61.1	61.4	63.7	57.3
Caeum: Empty Contents	1299.0	997.0	993.0	829.0	524.0	76.8	76.4	63.8	40.3
Rectum: Empty Contents	888.0	793.0	781.0	801.0	683.0	89.3	89.0	90.2	76.9
Kidneys (2)	2395.0	2707.0	2787.0	3539.0	2474.0	113.0	116.4	147.8	103.3
Psoas: Right Left	156.0	170.0	143.0	143.0	104.0	109.0	91.7	91.7	66.7
Brain	306.0	436.0	214.0	335.0	129.0	142.5	69.9	109.5	42.2
Eyes (2)	232.0	269.0	252.0	210.0	212.0	115.9	108.6	90.5	91.3
Tongue	39.0	125.0	230.0	235.0	234.0	320.5	589.7	602.5	600.0
Cord	310.0	203.0	231.0	256.0	231.0	65.5	74.5	82.6	74.5
Tendon	62.0	464.0	451.0	371.0	260.0	86.0	84.0	69.1	48.4
Skin of carcass	1157.0	1504.0	1711.0	1670.0	1434.0	130.0	147.9	144.3	124.0
Glands	5830.0	5798.0	5636.0	5198.0	4706.0	99.4	97.0	89.2	80.7
	234.0	213.6	227.4	166.0	175.0	1.3	97.2	74.8	

High-Plane pigs are kept to the same age as the Low-Plane, the brain weight is the same.

(b) *Eyes*. The eyes behaved similarly to the brain, increasing in weight during submaintenance to 111.5 % of the control. This was also noted by Manassein (1869) and by Jackson (1915) with young albino rats.

• (2) *Thoracic organs*

(a) *Heart*. The weight of the heart fell abruptly to 79.4 % of the control during the first 23 days of submaintenance and afterwards falls successively to 73.5, 71.8 and 69.9 % of the control during submaintenance periods of 51, 80 and 135 days respectively.

The initial drop in the weight of the heart may be due partly to loss of fat and partly to atrophy of the musculature during a period when the heart was doing less and less work as a result of decreased metabolic activity of the pigs under submaintenance conditions. Thus when the adjustment of the pig's metabolism to submaintenance conditions became more complete the rate of loss of weight of the heart decreased.

(b) *Lungs and trachea*. The lungs and trachea were affected similarly to the heart. After a marked initial drop, the weight remained fairly constant even after 135 days' submaintenance. As in the case of the heart the initial atrophy is probably due to the change from external to internal nutrition.

(3) *Abdominal organs*

(a) *Liver*. The weight of the liver fell to 48.4 % of the control during the first stages of submaintenance and remained at this level throughout the experimental period. A sudden loss in the weight of the liver in various species during the initial stages of inanition has been described by Manassein (1869), Bourgeois (1870), Lukianow (1892) and others. The discharge of glycogen, stored in the liver, as a result of the change from a supermaintenance to a submaintenance plane of nutrition is insufficient to account for the whole of the loss in weight, the glycogen content of the liver being only about 1 % of its weight (E. C. Smith, Low Temperature Research Station, private communication). It seems possible that some part of this loss is due to the discharge of storage proteins as described by Addis *et al.* (1936), and the remainder to loss of water.

(b) *Kidneys*. There was a loss in the weight of the kidneys of the submaintenance pigs of from 20 to 30 % of the control, although the weight of the kidneys varied somewhat irregularly.

(c) *Spleen*. The weight of the spleen is normally very variable, but

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even so there was a progressive loss in weight with increasing length of the submaintenance period with a reduction of 47.9 % of the control after 135 days.

Atrophy of the spleen and lymphoid tissue in general as a result of inanition has been observed by Bidder & Schmidt (1852), Manassein (1869) and others.

(d) *Pancreas.* There was a marked pancreatic atrophy, though individually the weight of pancreas was rather variable ranging from 74.4 to 48 % of the control.

(e) *Alimentary tract.* Previous workers, notably Bourgeois (1870), have observed losses in weight of the alimentary tract accompanied by thinning of the walls and shortening. Table 2 shows that the weight of alimentary tract not only decreases as a whole but the effect is most marked in the oesophagus and rectum. The stomach loss is intermediate between that of the oesophagus and small intestine. The large intestine loses more than the small intestine but less than the caecum, and the latter loses more than the rectum.

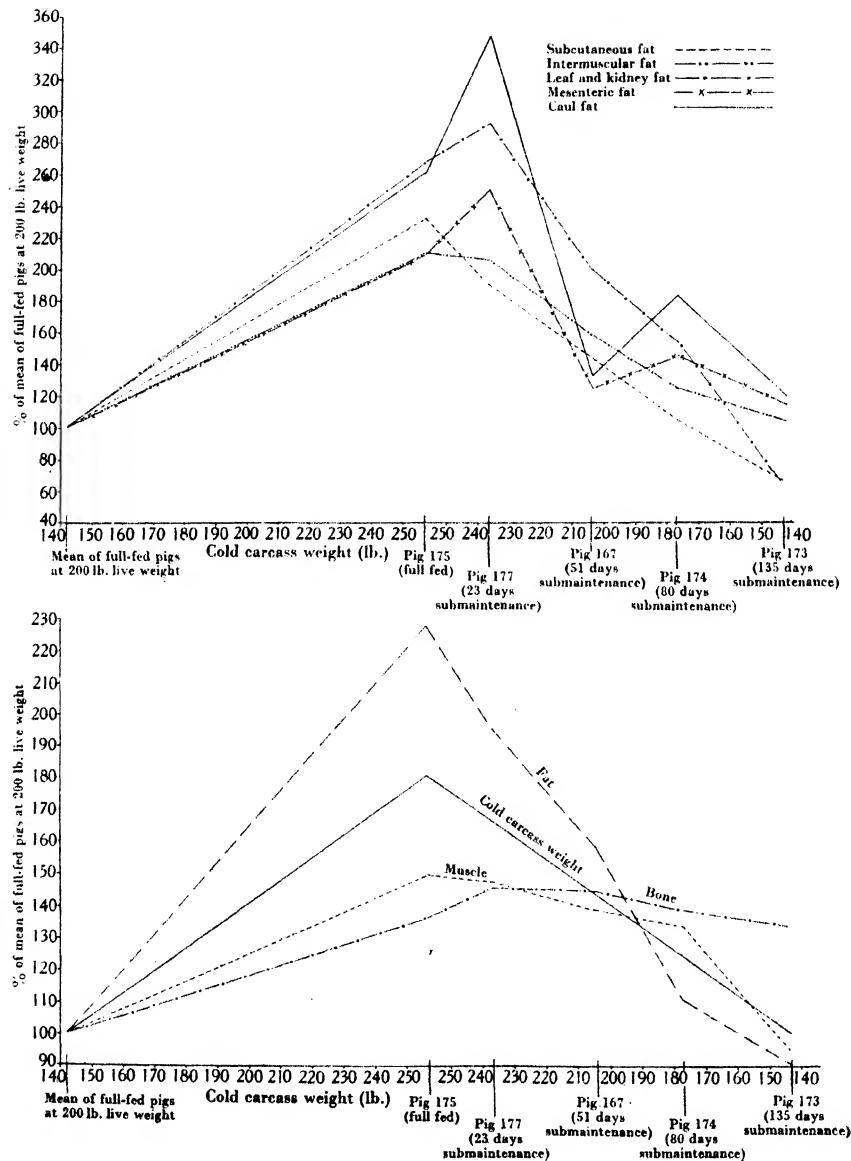
There was also pronounced thinning of the wall of the small intestine, and progressive shortening from 2289 cm. in pig 175 to 1786 cm. in pig 173.

EFFECT ON CARCASS COMPOSITION (Text-figs. 2, 3)

The effect of a submaintenance diet on the composition of the carcass is shown in Table 3 and Text-fig. 2.

(1) *Bone.* There was a tendency for bone to continue to grow in spite of the animal being subjected to a submaintenance diet, and only when the latter was prolonged (80-135 days) did any apparent loss in weight occur. This is confirmed by the results of Lukianow (1892), Bidder & Schmidt (1852), Falck & Scheffer (1854), Falck (1875), Bourgeois (1870), and Voit (1866, 1894).

When the various joints are considered individually it becomes apparent that growth rates of the bone in each is affected differentially. Thus growth in the lumbar vertebrae proceeds more slowly, and ceases earlier than in the head (Table 4). In spite of a certain amount of variation due mainly to differences in the number of sacral and thoracic vertebrae and ribs in the different pigs there is a fairly well-defined gradient in the effect on the bone of the various joints. Allowing for the variability already noted, the general trend is that the weight of bone of the joints has been affected in the inverse order to their order of development, that is to say, the early-developing head and legs have been penalized less than the late-maturing loin.



Text-fig. 2. Effect of submaintenance on fat depots.

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Table 3. *Composition of carcass*

	Pig 175 wt. (g.)	Pig 177 wt. (g.)	Pig 174 wt. (g.)	Pig 173 wt. (g.)	Pig 175 % control	Pig 177 % control	Pig 167 % control	Pig 174 % control	Pig 173 % control
Bone	9427.9	10089.3	10027.2	9606.6	9242.5	100	107.0	106.4	101.9
Muscle	37413.0	36346.0	34772.0	33508.0	23792.0	100	98.8	92.9	89.6
Fat	58254.0	50006.0	37970.0	28380.0	23203.0	100	85.8	65.2	48.7

Table 4. *Composition of joints*

	Pig 175 control wt. (g.)	Pig 177 wt. (g.)	Pig 167 wt. (g.)	Pig 174 wt. (g.)	Pig 173 wt. (g.)	Pig 177 % control	Pig 167 % control	Pig 174 % control	Pig 173 % control
Head:									
Bone	1801.0	2110.4	2044.0	1867.0	1944.7	117.2	113.5	103.7	108.0
Muscle	1313.0	1236.0	1093.0	1279.0	1049.0	94.1	83.2	97.4	79.9
Fat	2397.0	1691.0	1624.0	1448.0	1536.0	70.5	67.8	60.4	64.1
Neck:									
Bone	416.0	431.0	468.0	438.0	419.0	103.6	112.5	105.3	100.7
Muscle	3162.0	3763.0	3497.0	3519.0	2423.0	119.0	110.6	111.3	76.6
Fat	5986.0	5964.0	5442.0	4152.0	3505.0	99.6	90.9	69.4	58.6
Thorax:									
Bone	2102.0	2197.0	2172.0	2092.0	1933.0	104.5	103.3	99.5	92.0
Muscle	9363.0	9287.0	8633.0	8581.0	5401.0	99.2	92.4	91.6	57.5
Fat	19317.0	16814.0	11848.0	8862.0	6625.0	87.0	61.3	45.9	34.3
Loin:									
Bone	556.0	593.0	526.0	506.0	531.0	106.7	94.6	91.0	95.5
Muscle	3522.0	3404.0	2773.0	2714.0	1776.0	96.6	78.7	77.1	50.4
Fat	9388.0	6801.0	4375.0	3168.0	2388.0	72.6	46.7	33.8	25.5
Pelvis:									
Bone	769.0	793.0	718.0	708.0	707.0	103.1	93.4	92.1	91.9
Muscle	2288.0	2490.0	2149.0	2174.0	1897.0	109.8	94.8	95.9	83.6
Fat	4874.0	4678.0	3553.0	2396.0	2370.0	96.0	72.9	49.2	48.6
Shoulders (2):									
Bone	1975.4	2054.6	2114.4	2007.8	1887.8	104.0	107.0	101.6	95.6
Muscle	8033.0	7210.0	7178.0	7273.0	5090.0	89.8	96.5	90.5	63.4
Fat	7689.0	6277.0	5360.0	3848.0	3106.0	81.5	69.6	50.0	40.3
Legs (2):									
Bone	1808.5	1910.3	1848.8	1856.8	1820.0	105.6	102.2	102.7	100.6
Muscle	9752.0	9554.0	8857.0	7968.0	6165.0	98.0	90.8	81.7	63.1
Fat	8613.0	7780.0	5783.0	4506.0	3673.0	90.3	67.1	52.3	42.6

(2) *Muscle.* Table 4 and Text-fig. 3 show that the effect of a sub-maintenance diet on the weight of muscle in the various joints proceeds along the same general lines as in the case of bone. However, the tendency towards persistent growth of muscle in the early stages of sub-maintenance is much weaker than in the case of bone, and the final relative losses are much greater.

• The various joints are again differentially affected in the same order as described for the weights of bone.

(3) *Fat.* Although as Text-fig. 3 and Table 4 show the effect of sub-maintenance on the fat of the carcass is much more severe than in muscle or bone, the order in which the joints are penalized is exactly the same as before—the late-developing joints first and the early-developing joints last.

Not only is this the case but also a similar trend can be traced in the case of the three different depots of fat in the carcass. Subcutaneous fat, which is later developing than intermuscular fat, is penalized to a greater extent. On the other hand, the abdominal fats which are earlier developing than subcutaneous fat are not so adversely affected. In addition, the subcutaneous and intermuscular fat of the late-developing joints suffer greater losses in weight than the corresponding fats of the early-developing joints.

Summarizing the tissues, muscle fat and bone are affected by a sub-maintenance diet in reverse order to their order of development, i.e. the early-developing bone is penalized less than the late-developing fat with muscle intermediate.

These results for a submaintenance plane of nutrition are in striking agreement with McMeekan's work on supermaintenance planes of nutrition on the carcass composition of the pig, in which he showed that supermaintenance planes of nutrition affect the tissues, muscle, fat and bone, in the carcass as a whole in the order of their development. In addition, each tissue is affected more in the late-developing joints such as the loin than in the early-developing joints such as the head.

(4) *Comparisons with McMeekan's High- and Low-Plane pigs.* In Table 5 and Text-fig. 3 the weight of the various parts of the carcass have been expressed as a percentage of the corresponding parts of the High Plane of nutrition pigs (McMeekan) at 200 lb.

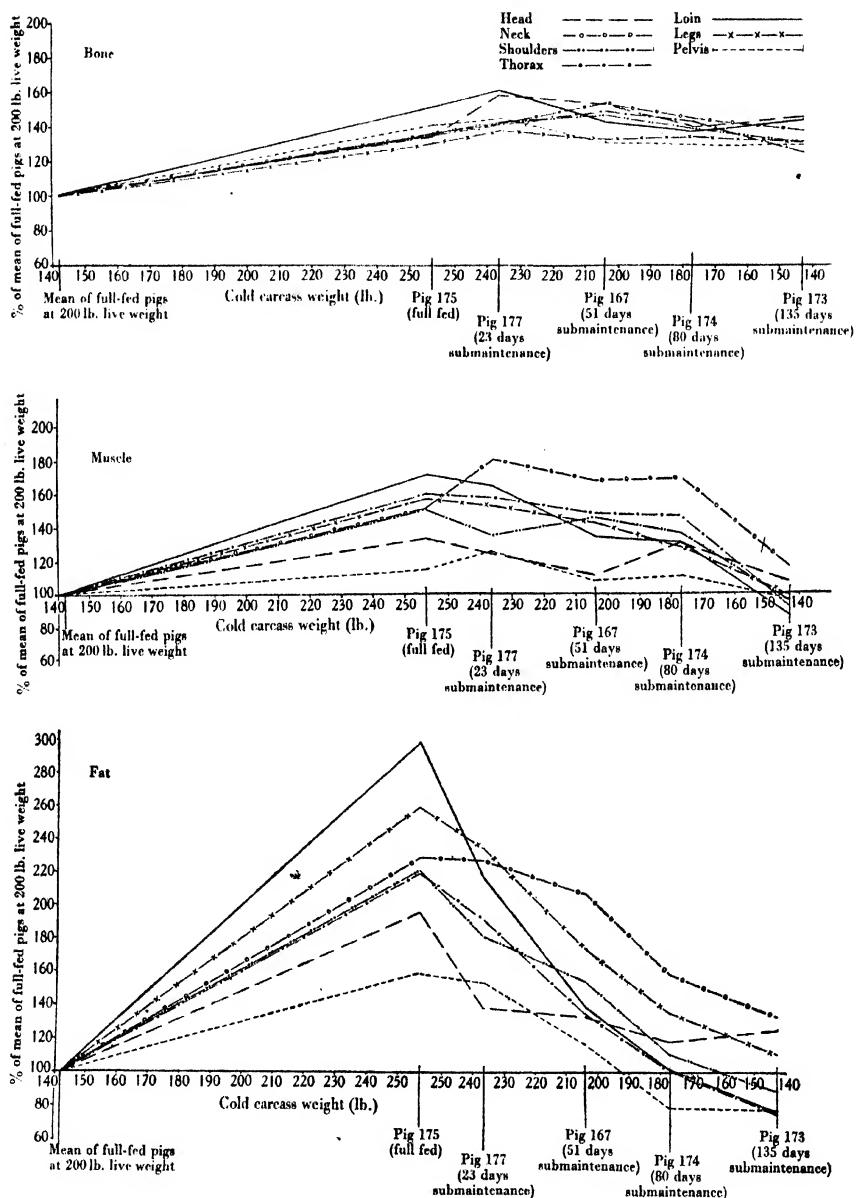
They show the relative changes in the weight of organs and in bone, muscle and fat in the carcass as a whole and in the separate joints, first on a supermaintenance plane of nutrition and then on a submaintenance plane.

Table 5. Comparison with High-Plane males (McMechan)

	High-High (McMechan)	Pig 175	Pig 177	Pig 167	Pig 174	Pig 173	Pig 175 % H.H.	Pig 177 % H.H.	Pig 167 % H.H.	Pig 174 % H.H.	Pig 173 % H.H.
Days on submaintenance	—	—	—	—	—	—	—	—	—	—	—
Age at slaughter (days)	159	241	23	51	80	135	—	—	—	—	—
Carcass weight (g.)	64570	116575	102726	92334	80287	64865	180-54	166-14	143-31	124-34	100-46
Composition of carcass:											
Bone	6930	9427-9	10089-3	10027-2	9606-6	9242-5	136-04	145-59	144-69	138-62	133-37
Muscle	25059	37413	36946	34772	33508	23792	149-30	147-44	138-76	133-72	94-94
Fat total	25504	58254	50006	37970	28380	23203	227-64	195-41	148-38	110-90	90-67
Subcut	19177	44511	36499	27079	20210	16413	232-11	190-33	144-33	105-39	85-59
Intermuscular	65111	13743	13507	10291	8170	6790	211-07	207-45	158-06	125-48	104-29
Head: Total	5042	7500	7286	7117	6641	6540	—	—	—	—	—
Bone	1338	1801	2110-4	2044	1867	1944-7	134-60	157-73	152-77	139-54	145-34
Muscle	985	1313	1236	1093	1279	1049	133-30	125-48	110-96	129-85	106-50
Fat	1224	2397	1691	1624	1448	1536	195-83	138-15	132-68	118-30	125-49
Neck: Total	5491	10367	10841	10290	8756	6985	—	—	—	—	—
Bone	304	416	431	468	438	419	136-84	141-78	153-95	144-08	137-83
Muscle	2089	3162	3763	3497	3519	2423	151-36	180-13	167-40	168-45	115-99
Fat	2624	5986	5964	5442	4152	3505	228-13	227-29	207-39	188-23	133-57
Shoulders (2): Total	11310	19310	17100	16830	14500	11398	—	—	—	—	—
Bone	1444	1975-4	2054-6	2114-4	2007-8	1987-8	136-8	142-29	146-43	139-04	130-73
Muscle	5341	8033-0	7210	7748	7273	5090	150-4	134-99	145-07	136-17	95-30
Fat	3481	7699	6277	5360	3848	3106	221-17	180-32	153-98	110-54	89-23
Thorax: Total weight	17661	33016	30149	24468	21104	16259	—	—	—	—	—
Bone	15560	2102	2197	2308	2223	1933	135-61	141-74	148-90	143-42	124-71
Muscle	5861	9363	9287	8653	8581	5401	159-75	158-45	147-64	146-41	92-16
Fat	8809	19317	16814	11848	8882	6625	219-29	190-87	134-50	100-80	75-21
Loin: Total weight	6115	14641	11712	8489	6992	5338	—	—	—	—	—
Bone	368	556	593	526	506	531	151-09	161-14	142-93	137-4	144-29
Muscle	2063	3522	3404	2773	2714	1776	170-72	165-00	134-42	131-56	86-09
Fat	3139	9368	6801	4975	3168	2388	298-44	216-66	139-38	100-92	76-08
Pelvis: Total	61095	8752	8662	7046	5843	5595	—	—	—	—	—
Bone	5446	769	793	718	708	707	140-84	145-24	131-50	128-67	129-49
Muscle	1974	2268	2490	2149	1897	114-89	126-14	108-87	110-13	96-10	—
Fat	3053	4874	4678	3553	2396	2370	159-65	153-23	116-38	78-48	77-63
Legs (2): Total weight	12147	22370	21320	18725	16470	13524	—	—	—	—	—
Bone	1385	1808-5	1910-3	1848-8	1866-8	1820	130-58	137-93	133-49	134-06	131-41
Muscle	6221	9554	8857	7988	6156	156-76	153-58	142-37	128-08	98-96	—
Fat	3325	8613	7780	5783	4506	3673	259-04	233-98	173-92	135-52	110-47

				Internal fat									
Leaf and kidney fat	1511	4057	4426	3022	2331	1005	268-50	292-92	200-00	154-27	68-51	68-51	
Caul fat	129	338	449	172	237	155	262-02	348-06	133-33	183-72	120-16	120-16	
Mesenteric fat	1075	2266	2696	1351	1568	1242	210-79	250-79	125-67	145-86	115-53	115-53	
							Organs (including blood, etc.)						
Blood	4083	4877	4227	4825	3920	3746	119-45	103-53	118-17	96-01	91-75	91-75	
Skin and hair	537	872	1225	960	1180	1023	162-38	228-12	178-77	219-74	190-53	190-53	
Hoofs: Fore	43	61	61	69	70	60	141-86	141-86	160-47	162-79	139-53	139-53	
Hind	30	47	44	54	49	47	156-67	146-67	180	163-33	156-67	156-67	
Bladder (empty)	53	64	41	61	46	45	120-75	77-36	115-09	86-79	84-91	84-91	
Neck thymus	—	46	94	98	14	17	—	—	—	—	—	—	
Heart thymus	—	39	47	34	10	10	—	—	—	—	—	—	
Gall bladder (full)	34	55	18	47	16	34	161-76	52-94	13-82	47-06	100-00	100-00	
Diaphragm	414	546	542	483	583	396	131-88	130-92	116-67	140-82	95-65	95-65	
Pancreas	119	125	73	93	75	60	105-04	61-34	78-15	63-03	50-42	50-42	
Pericardium and blood vessels	191	506	468	277	478	288	264-92	245-03	145-03	250-26	150-79	150-79	
Heart (without pericardium and blood vessels)	283	408	324	300	293	285	144-17	114-49	106-01	103-53	100-71	100-71	
Lungs and trachea	632	935	790	744	799	773	147-94	125-00	117-72	126-42	122-31	122-31	
Liver	2520	2297	1112	1147	1055	1046	91-15	44-13	45-52	41-87	41-51	41-51	
Spleen	83	163	116	116	102	78	196-39	139-76	122-89	93-98	93-98	93-98	
Kidneys (2)	425	310	203	231	256	231	72-94	47-76	54-35	60-24	54-35	54-35	
Brain	99	102	103	115	123	136	103-03	104-04	116-16	124-24	137-37	137-37	
Eyes (2)	11	13-1	12-7	13-3	14-5	14-6	119-09	115-45	120-91	131-82	132-73	132-73	
Tongue	157	197	216	248	212	213	—	—	—	—	—	—	
Cord	—	62	84	84	78	78	—	—	—	—	—	—	

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Text-fig. 3. Effect of submaintenance on composition of joints.

At the end of the supermaintenance period fat was growing most rapidly, then muscle and then bone. On changing to submaintenance the order was reversed, fat was lost most quickly, then muscle, while bone actually continued to grow for a time. The same tendency is apparent in the tissues within the various joints, i.e. bone, muscle and fat are affected more in the loin than in the head.

Compared with McMeekan's Low Plane of nutrition pigs at the same carcass weight (143 lb.), pig 173, after 135 days' submaintenance, contained 17 % more bone, 5 % less muscle and 26 % more fat. In the Low-Low pigs the plane of nutrition was so low that the deposition of fat was practically inhibited, while bone and muscle made slow but definite growth. While pig 173 was on a supermaintenance diet, bone, muscle and fat continued to grow well, so that when the change-over to submaintenance was made it had already accumulated large reserves of muscle and fat and was able to survive 135 days' submaintenance and still retain more fat and about the same amount of muscle as the Low-Low pigs at the same carcass weight. The difference between the submaintenance and McMeekan's Low-Low pigs is most marked in the joints which were most developed when the change-over to a submaintenance diet was made. At this stage the loin was the least developed joint, and as it suffered the greatest losses during submaintenance its final composition approximated to that of the loin in the Low-Low pigs.

EFFECT ON CARCASS MEASUREMENTS

Table 6 and Text-figs. 4 and 5 show the effect of submaintenance on carcass measurements.

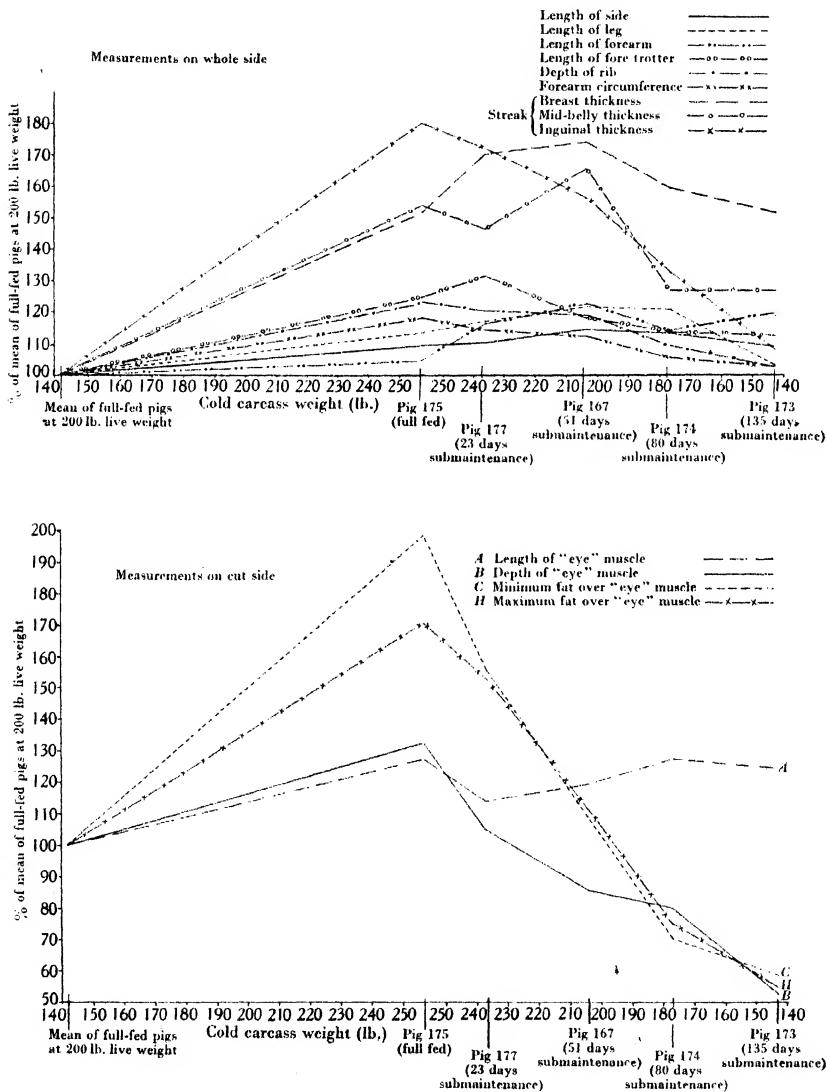
Length of side. The length of side appears to increase during submaintenance, but this is complicated by the fact that pigs 167 and 174 had sixteen thoracic vertebrae whereas the others only had fifteen. Allowing for this variation, it appears that the length of side is substantially unaltered as a result of submaintenance.

Thickness of streak. As a rule the thickness of streak is increased on fasting as a result of the shortening of the muscle of the body wall consequent upon a reduction in the volume of the gut content. In the present experiment the initial thickening of the streak has been superseded by a thinning due to the loss of subcutaneous and intermuscular fat. The effect was accentuated in the flank region by the distension of the body wall resulting from the ingestion of straw.

Back fat. The thickness of back fat in the loin is reduced more than the thickness at the shoulder and the rump, which is in line with the effect

Table 6. Effect of submaintenance on carcass measurements

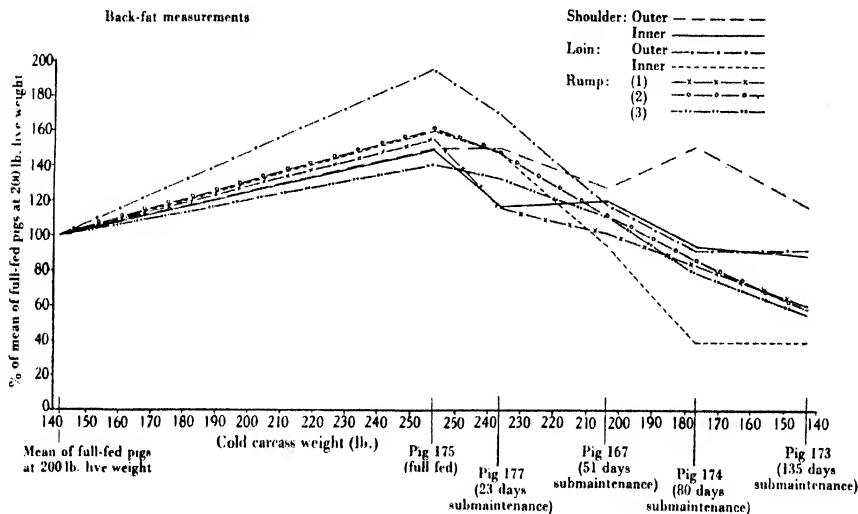
on the total fat of the loin, thorax and pelvis respectively. The thickness of the inner and the outer layers of back fat in the shoulder and loin regions, where they may be clearly defined, was also measured. The inner



Text-fig. 4. Effect of submaintenance on carcass measurements.

layer of back fat is deposited at a later stage than the outer layer, and is more essentially a fat-storage depot, as is shown by the fact that it suffers relatively greater losses in a submaintenance period than the outer layer. At the same time the inner layer of back fat is more severely affected on the loin than at the shoulder.

Bone. The length of bones is, in general, unaffected by submaintenance, so that the relation between the length of any particular bone or combinations of bones and the total weight of bone in the carcass no longer holds under conditions of prolonged under-nutrition when the



Text-fig. 5. Effect of submaintenance on back-fat measurements.

weight of bone decreases but the length is unaffected. The combined weight of the four cannons which are removed from the carcass in butchering do not show a close relation to the weight of bone in the whole carcass, although McMeekan found a good correlation in his more numerous supermaintenance animals.

From the measurements of the lengths of various bones it seems probable that the loss in weight of bone during prolonged submaintenance is only an apparent one, due to pig 173 having naturally shorter bones than the others.

Muscle. The length of the eye muscle (*A*) which is an early-maturing character is less affected than the depth (*B*) which is late-maturing. The depth of eye muscle is a commercially important character, so the effect of starvation upon it is important.

The curve showing the effect of submaintenance on the *B* measurement follows the same general trend as that for the total muscle, but the relation between the decrease in *B* and the decrease in total muscle does not appear to be a very close one.

Fat (C). The *C* measurement, thickness of fat over the eye muscle, was shown by McMeekan to be the best single measure of the total fat in the carcass. In the present experiment the relation between *C* measurement and the total fat was still very close, confirming its value as an index of the total quantity of fat.

(*H*) The *H* measurement also bears a fairly close relationship to the total fat in the carcass, but it is not so useful in this connexion as *C*.

In general, the experimental results suggest that measures of later-maturing characters such as *B*, the thickness of the eye muscle and *C*, the fat over it, are better indices of development than early measures such as *A*, the length of eye muscle and the shoulder back fat.

DISCUSSION

When pigs are fed on a submaintenance diet the parts of the body are stunted or reduced in reverse order to their order of development, that is to say, the latest developing parts such as the loin are most affected. Thus the wave of growth described by McMeekan is to a large extent reversed, though bone may continue to grow for a time. Although there is persistent growth of bone, the growth rate of the later developing bones, such as the lumbar vertebrae, is reduced to a greater extent than that of early-maturing bones such as the skull. As a result of the reversal of growth gradient the body form reverts to the juvenile type in which a large proportion of the weight is in the fore end (Pl. 1, figs. 1, 3).

As regards the bearing of the results on the evaluation of carcass quality and on commercial feeding practice the first point of interest is the relation between carcass measurement and carcass composition. Such measurements as have already been shown to be closely related to the weights of muscle and fat, but not bone, in the carcass of pigs fed on a supermaintenance plane of nutrition appear to maintain the same relationship under submaintenance conditions, which gives additional support to their use in estimating the carcass composition of pigs reared under widely differing nutritional conditions.

It has long been a common practice, notably in the case of beef cattle but also to some extent in the case of pigs and sheep, to subject growing animals to a "store" period of varying intensity. According to the

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duration and severity of the store period the animal makes slow growth, maintains its weight or actually loses weight before being put up to fatten for marketing. The determination of the effect of a store period on the carcass composition at marketing weights is a problem of considerable importance. A similar problem exists in countries where food supply is determined by climatic factors such as rainfall, when animals grow fat after the rains when food is abundant and they lose weight in the succeeding drought.

The results of the present experiment show that loss of body weight consists mainly of a loss of fat together with some loss of muscle. The permanence of these effects has now to be determined, for if the loss in muscle is replaced during a subsequent period of good nutrition no permanent loss in carcass quality will result. On the other hand, if, under certain conditions, there is a permanent loss of muscle and subsequent growth under good nutritive conditions consists mainly of fat, the carcass will tend to be too fat. In this connexion it may be said that a common fault in bacon carcasses is that the "eye" muscle instead of being more or less full and round often has a pronounced kink in the top. It may well be that adverse conditions of nutrition at a period in the pig's life lead to a more or less permanent decrease in size of the muscle fibres, and when fattening is resumed rapid deposition of fat above the muscle forces it into this kinked shape.

It is, therefore, desirable to extend present investigation by growing pigs to bacon weight on a High-Low-High Plane of nutrition and determining the effect on the carcass composition.

SUMMARY

Five inbred Large Whites from the same strain as used by McMeekan (1940) were reared on a High Plane of nutrition to approximately 330 lb. live weight at which one was killed as a control. The rest were put on to a submaintenance diet of straw and water and killed successively at roughly equal intervals in live weight between 330 and 200 lb. The weights of blood, organs and offals were determined and the carcasses were jointed and completely dissected into their constituent tissues. The total weights of each and the weights within the various joints were recorded.

(1) *Organs.* The early-maturing organs, brain, eyes, etc., continued to grow. Other organs like the heart, liver, lungs, etc., suffered a greater or less degree of atrophy which was probably determined by a suspension of their functions.

(2) *Carcass composition.* The tissues of the carcass were affected in reverse order to their development, i.e. fat most, muscle less and bone least. Bone continued to grow in the earlier stages of submaintenance.

The joints were also affected in inverse ratio to their order of development. Within the fat depots the later developing kidney fat reduced first, then the subcutaneous fat and lastly the early-developing intermuscular, caud and mesenteric fat.

Within the subcutaneous fat the later-maturing inner layer is affected more than the early-maturing outer layer.

I wish to express my thanks to Dr J. Hammond, F.R.S., for his help and advice throughout the experiment; to the willing band of helpers who did the dissections and to Mr C. Williamson for the photographs. I am also indebted to Dr Callow and Dr E. C. Smith of the Low Temperature Research Station for permission to use some of their data.

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POSTSCRIPT

Maintenance requirements of pigs between 200 and 300 lb. live weight

As the pigs in this experiment received no food other than straw and water, the energy required for maintenance must have been derived from the body muscle and fat. The only requirements for energy of pigs on a submaintenance ration are for maintenance and movement, so it is reasonable to suppose that, if the actual loss of pure fat and protein can be calculated and expressed in therms, the resulting figure will represent the minimum energy requirements for maintenance and movement.

In order to do this it is necessary to know the percentage of fat in the fatty tissues of the individual pigs, percentage fat in the muscles and the composition of the organs. Dr Callow was able to supply the figures for the percentage fat in the fatty tissues, but the muscle samples have not yet been analysed. Hence an estimate of the loss of intramuscular fat has been arrived at as follows. The loss in weight of the fatty tissues amounted to 2, 7, 10 and 34 % of the control in pigs 177, 167, 174 and 173 respectively. From figures supplied by Dr Callow it appears that normal pig muscle contains about 10 % of fat, so to estimate the loss in intramuscular fat, the weight of intramuscular fat in the control pig was assumed to be 10 % of the weight of muscle and 2, 7, 10 and 34 % of this figure were taken to represent the loss of intramuscular fat in the remaining pigs.

No figures were available for the composition of the organs, so the loss of weight of the organs was divided between muscle and fat in the proportion of the muscle to fat ratio in the carcass.

The figures obtained for total loss of fat per day were then expressed in therms on the basis of 100 lb. pure fat = 422 therms, and the results are contained in the table below.

As no data are available concerning the chemical composition of the muscle, the only way in which the protein requirements can be expressed is to take the figure given in Maynard's *Animal Nutrition*, 2 mg. protein per Cal.

The maintenance requirements of pigs weighing 200, 250 and 300 lb. are given by Morrison in *Feeds and Feeding* as 4.8-5.9, 5.4-6.4 and 5.9-7.0 therms per day respectively, which are considerably in excess of the figures calculated here, even allowing for the assumptions made in calculating these figures and the small number of pigs concerned.

(Received 26 August 1940)



Fig. 2.

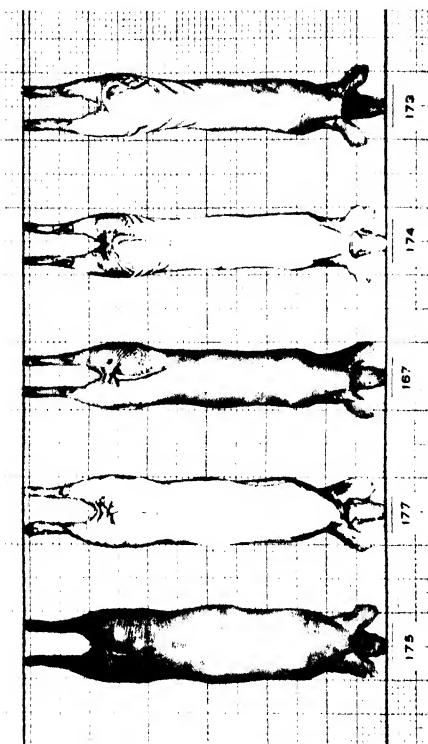


Fig. 3.

Fig. 1. Effect of submaintenance on body proportions. All to same shoulder-trotter height.

Fig. 2. Effect of submaintenance on loin cut. All to same eye-muscle length.

Fig. 3. Effect of submaintenance on body proportions. All to same total length.

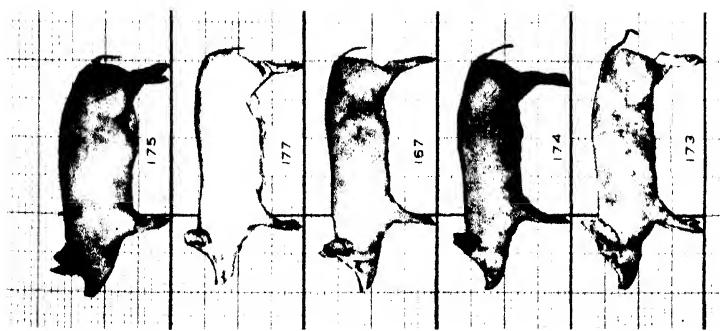


Fig. 1.

Final live weight ...
Days on submaintenance ...
% fat in fatty tissue ...

Pig 177
302 lb.
23
91.1

	Loss of fatty tissue per day g.	Loss of pure fat per day g.	Maintenance required therm	Protein required g.	Loss in wt. g.	Loss of fatty tissue per day g.	Loss of pure fat per day g.	Maintenance required therm	Protein required g.
Carcass fat depots	8,248	—	—	—	20,284	—	—	—	—
Intramuscular fat	74	—	—	—	259	—	—	—	—
Internal fat	—910	—	—	—	2,216	—	—	—	—
Fat in organs	2,248	—	—	—	2,419	—	—	—	—
Total	9,660	420.0	382.6	3.55	44.4	25,178	493.7	446.3	4.14

Final live weight ...
Days on submaintenance ...
% fat in fatty tissue ...

Pig 174
234 lb.
80
87.9

	Loss of fatty tissue per day g.	Loss of pure fat per day g.	Maintenance required therm	Protein required g.	Loss in wt. g.	Loss of fatty tissue per day g.	Loss of pure fat per day g.	Maintenance required therm	Protein required g.
Carcass fat depots	29,874	—	—	—	35,051	—	—	—	—
Intramuscular fat	370	—	—	—	1,258	—	—	—	—
Internal fat	2,626	—	—	—	2,090	—	—	—	—
Fat in organs	2,398	—	—	—	2,398	—	—	—	—
Total	35,267	440.8	387.5	3.60	45.00	40,797	302.2	258.1	2.40

Pig 167
263 lb.
51
90.4

Pig 173
188 lb.
135
85.4

DRAINAGE AND EVAPORATION FROM FALLOW SOIL AT ROTHAMSTED

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(With Nine Text-figures)

THE three drain gauges at Rothamsted were built in the summer of 1870. As details of their construction were given by Lawes *et al.* (1881) and by Miller (1906), it is sufficient here to recall that they each enclose a block of undisturbed soil 1/1000 acre (4 m.²) in area kept free of vegetation; the three blocks of soil are respectively 20 in. (0.5 m.), 40 in. (1 m.) and 60 in. (1.5 m.) deep. Daily readings have been taken of the drainage which passes through the perforated plates which support the soil blocks, and since 1925 these daily readings have been supplemented by automatically recording apparatus installed by Dr Keen. The rain and drainage waters have been analysed for nitrate and chloride.

The monthly totals of rainfall and drainage have been published regularly in the *Annual Reports* of the Station. Gilbert (1891) reproduces these data up to 1891, Scott (1900) up to 1899, and Miller gives the yearly totals and monthly means to 1905.

The main conclusions reached by Lawes *et al.* from an examination of the records for the first ten years were:

- (1) There is no great difference between the records for the three gauges, and, consequently, no marked effect due to depth of soil. The 40 in. gauge may be slightly defective.
- (2) The mean annual drainage is about half the mean annual rainfall.
- (3) The annual drainage tends to be a greater fraction of the annual rainfall in years of high rainfall. Hence the annual drainage is more variable than the annual rainfall.
- (4) The annual deficit¹ differs little from year to year.
- (5) The deficit for the period October to March inclusive is generally less than one-third of the annual deficit, and agrees substantially with the evaporation from an open water surface (Greaves, 1876).

¹ The deficit for any period is the difference between the rainfall and the drainage. The precision with which the deficit is a measure of evaporation is discussed below.

(6) The deficit for the period April to September is less than the evaporation from an open-water surface, presumably because the soil surface is often dry.

(7) In January the mean monthly drainage is greatest in relation to the mean monthly rainfall; in July it is least.

(8) The drainage in any period must depend on the way in which the rain is distributed as well as on its total amount.

(9) The few cases where a month's drainage had exceeded its rainfall were due to snow or frost at the end of the previous month.

(10) All the chloride in the drainage water is brought into the soil by the rain. A considerable part of the nitrate is produced in the soil.

(11) The fluctuations in the chloride and nitrate concentrations indicate that the drainage takes place mainly through root-holes, worm-holes and fissures in the soil.

Since 1881 a number of additional observations and deductions have been recorded.

Warington (1900) noticed that the annual deficit had tended to decline. He thought this might be because stones had accumulated at the surface and were hindering evaporation. In a note on the review of the data by Scott, Gibbs (1904) suggested several possible causes: a decrease in the annual sunshine, an increase in the proportion of winter rain, a change in the physical state of the soil. The last possibility was further considered by Russell (1907).

Miller gave a table from which it appears that the annual deficit tends to be slightly greater in years of large rainfall, but he did not draw attention to this fact. He also pointed out that the 60 in. gauge tends to give slightly more drainage than the 20 in. gauge from January to May, and slightly less during the rest of the year, but offered no explanation.

To the monthly means of drainage (average for the three gauges) Crowther (1930) fitted a regression equation involving the mean monthly rainfall and the mean monthly air temperature and obtained significant correlations with both. He hoped this might approximate to a general expression for the dependence of drainage on rainfall and temperature.

Koshal (1934) made a statistical study in which the data for each gauge and each month were examined separately. He obtained thirty-six regression equations connecting the drainage in any one month with the rainfall and mean air temperature of that month. The regression coefficients on rainfall vary from nearly unity in January to about 0.5 in July. Thus an extra inch of rain falling in January produces almost an inch

more drainage, whereas an extra inch in July gives only about half an inch extra drainage.

To Koshal's surprise the regression coefficients on mean air temperature were small and mostly non-significant. Two, indeed, were positive, whereas he had expected that a rise in temperature would diminish drainage by increasing evaporation. His analysis left the greater part of the variation in drainage from month to month unexplained. He therefore suggested that an important part of this variation might be due to an annual cyclic change in the water content of the gauges.

Koshal obtained factors for the time variation of the constants in his equations but none was significant. Thus this very full statistical analysis failed to demonstrate any progressive change in the physical condition of the soil.

EXAMINATION OF THE AUTOMATIC RECORDS OF RAINFALL AND DRAINAGE

A. *Drainage after rain*

The drainage residue.

The automatic records show that drainage, when it occurs, continues after the rain which causes it has stopped. During these periods the drainage rate steadily decreases, becoming vanishingly small in 24 hr. in the case of the 20 in. gauge, and in about 48 hr. in the case of the 60 in. gauge. In the absence of further rain the amount of drainage still to come at any time in the drainage period we shall call the drainage residue.

Influence of temperature on drainage rate.

In a preliminary study of drainage after rain, measurements were made from the automatic charts of the drainage residue when the drainage rate had fallen to 0.0346 in. per hr. This rate corresponds to a 30° slope on the automatic charts and was selected because it is about the highest rate of drainage normally occurring after rain has stopped. The results for the 20 in. gauge shown in Table 1 cover all the cases from 1926 to 1939 in which rain stopped shortly before the drainage rate fell to 0.0346 in. per hr. and there was no more rain for 24 hr. In order to increase the number of observations, and thereby obtain better monthly averages, occasions were also included when additional rain not exceeding 0.05 in. fell soon after the 30° point. In these cases the drainage residue was obtained by subtracting the additional rain from the measured drainage.

Table 1 shows plainly that there is a seasonal variation of the drainage residue for a given drainage rate. Temperature must affect the rate of drainage through its influence on the viscosity of the percolating water. If, apart from the effect of temperature, the soil moisture conditions are always the same for the drainage residue, then the rate of drainage for a given drainage residue must be inversely proportional to the viscosity. Owing to the fortunate circumstance that the drainage curves on the charts are nearly exponential during the later stage of residual drainage, the drainage residues for a given drainage rate are almost inversely proportional to the drainage rates for a given drainage residue, i.e. the drainage residues (30° point) should be proportional to the viscosity.

Table 1. *Drainage residue beyond 30° point (20 in. gauge)*

Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
0.13	0.13	0.12	0.12	0.11	0.07	0.07	0.09	0.09	0.10	0.12	0.13
0.14	0.15	0.14	0.12	0.12	0.09	0.10	0.10	0.09	0.12	0.13	0.14
0.14	0.15	—	0.12	0.12	0.09	0.10	0.10	0.10	0.12	0.13	0.14
0.16	0.16	—	0.13	0.12	0.09	0.11	0.11	0.10	0.13	0.14	0.14
0.16	0.17	—	0.13	0.13	0.09	0.11	0.11	0.12	0.14	0.14	0.18
0.16	0.18	—	0.15	0.14	0.10	0.11	0.11	0.12	0.14	0.14	0.18
0.17	—	—	—	0.16	0.11	0.12	0.12	0.12	0.14	0.15	—
—	—	—	—	—	0.11	0.12	0.13	0.13	0.15	0.16	—
—	—	—	—	—	—	0.12	—	0.13	0.17	0.16	—
—	—	—	—	—	—	—	—	0.13	—	0.16	—
—	—	—	—	—	—	—	—	—	—	0.16	—
—	—	—	—	—	—	—	—	—	—	0.16	—
—	—	—	—	—	—	—	—	—	—	0.18	—
Mean 0.150	0.155	0.130	0.130	0.130	0.095	0.105	0.110	0.115	0.135	0.150	0.150

We have no measurements of temperature in the soil of the drain gauges but readings are taken daily (9 a.m.) at 4, 8, and 12 in. under bare soil nearby. The temperatures at 1 ft. were abstracted for each of the occasions and averages were taken by months. The corresponding average viscosity is plotted for each month on the same graph as the average drainage residues, the scales being adjusted so that the grand mean viscosity has the same ordinate as the grand mean drainage residue (Fig. 1).

Since soil temperature fluctuates daily and depends on depth, the 9 a.m. reading at 1 ft. is only a rough guide to the temperature of the water that percolated through the soil of the gauge. The variable time relationship will be largely smoothed out in the monthly averages and the errors so caused should be randomly distributed. There will also be systematic differences between the means of the observed soil temperatures and the proper mean temperature of the percolating water which may

itself have a seasonal variation. We, therefore, conclude that the phase and amplitude of the seasonal variation in drainage residue are largely accounted for by viscosity variations. The discrepancies of Fig. 1 are of a kind which suggest a slight change in physical structure between the dry months of spring and summer and the wet months of winter, but they are not large enough to indicate any appreciable increase in the water retaining capacity of the soil between these seasons.

It was thought that evaporation might check residual drainage. If this occurred there would be a tendency for residual drainage to be greatly dependent on the meteorological conditions during the first few hours after rain stopped. No such effect has been found, and we conclude that evaporation occurring after a fall of rain has no measurable effect on the drainage response to that rain.

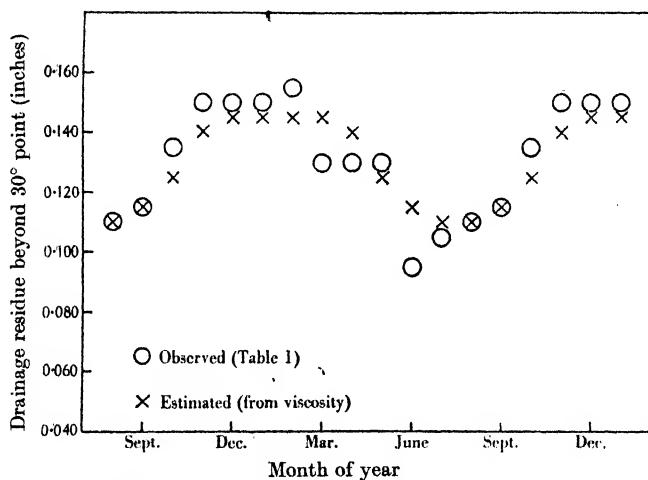


Fig. 1. Seasonal variation of drainage residue (20 in. gauge).

Maximum drainage rates.

There have only been a few occasions since 1925 where the automatic records show the attainment of a maximum rate of drainage. On 21 June 1936 0.90 in. of rain fell between 1.45 and 2.25 p.m. and a further 0.02 in. fell at 2.40 p.m. The 20 in. gauge was still draining at 5.00 p.m. when another violent storm broke. [The drainage rate had decreased to 0.0346 in. per hr. by 4.00 p.m.] From the June observations (Table 1 above) it follows that, had there been no more rain, only 0.095 in. more drainage would have been delivered after 4.00 p.m. This enabled us to find the drainage residue at times before 4.00 p.m.

In the case under consideration the drainage rate was at a maximum shortly before 2.25 p.m. when rain stopped. It seemed permissible during this period to obtain the drainage residue by subtracting the rain still to come. This procedure assumes that rain falling at this time added an equal amount to the drainage. The figures are given in Table 2 and the results are plotted in the top curve of Fig. 2.

Table 2. *Drainage rate and drainage residue (20) 21 June 1936*

Time p.m.	Rain to come (in.)	Drainage to come (in.)	Residue ($D - R$)	dD/dt (in. per hr.)
3.58	0.000	0.095 + 0.000*	0.095	0.035
47	0.000	+ 0.011	0.11	0.047
09	0.000	+ 0.050	0.15	0.095
2.50	0.000	+ 0.094	0.19	0.19
44	0.000	+ 0.113	0.21	0.26
34	0.020	+ 0.173	0.25	0.49
31	0.020	+ 0.202	0.28	0.70
28	0.025	+ 0.247	0.32	1.00
25	0.030	+ 0.303	0.37	1.00
21	0.085	+ 0.358	0.37	1.00
17	0.150	+ 0.416	0.36	1.00
14	0.230	+ 0.447	0.31	—
2.05	0.475	+ 0.453	0.07	—

* From Table 1.

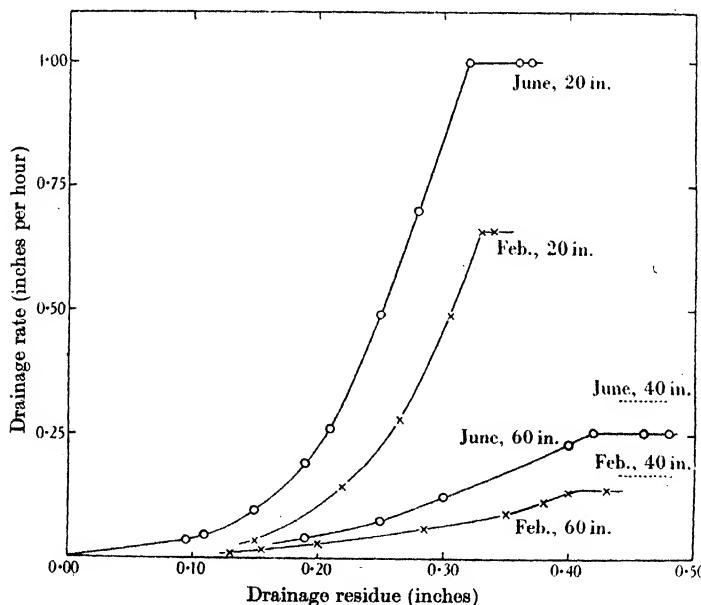


Fig. 2. *Drainage residue and drainage rate.*

It will be seen that the drainage rate was constant at 1 in. per hr. for drainage residues greater than 0.32 in. When the drainage residue exceeded this amount, the soil was evidently waterlogged, the amount in excess of 0.32 in. lying in puddles on the surface where it did not influence the drainage rate. In the case of the 60 in. gauge the maximum rate of 0.25 in. per hr. occurred for drainage residues greater than 0.43 in. The maximum rate for the 40 in. gauge was 0.32 in. per hr., but residual drainage from this gauge was augmented by a leak (p. 93) and so was not determined.

The downpour which occurred later in the same day precipitated 1.5 in. between 5.30 and 5.50 p.m. We have estimated that for some minutes the water was 1 in. deep on the soil of the gauges. During this time the drainage rate was 1.44 in. per hr. in the case of the 20 in. and 0.58 in. per hr. in the case of the 40 in. gauge. The pen of the 60 in. gauge failed to mark. These rates are appreciably higher than the maximum rates in Fig. 2. The probable explanation is that water deep enough to form a continuous sheet can flow sideways and feed any place where drainage is particularly easy. The periphery of the gauge where the soil block lies against the retaining wall provides a likely channel for the extra rapid drainage. Unfortunately the automatic equipment cannot record more than 1 in. of drainage in a day. Consequently the drainage residues after this flooding are not obtainable.

The other two curves in Fig. 2 were obtained from the records for 25 February 1937. The maximum drainage residues were only 0.34 and c. 0.42 in. for the 20 in. and 60 in. gauges respectively and the flat parts of the curve are very short. Support for the conclusion that on this occasion also the soil was waterlogged is obtained from inspection of the charts. During the period when the drainage curve was straight a small amount of rain fell at a nearly infinite rate and had no effect on the drainage slope.

On about a dozen occasions the drainage rate for the 20 in. gauge has exceeded 0.3 in. per hr. the maximum drainage residues ranging from 0.22 to 0.32 in. The 20 in. rate reached 0.82 in. per hr. on 9 October 1935 (i.e. midway between the June and February maxima), but owing to an uncertainty in the synchronization of the rainfall and drainage charts the value of the corresponding drainage residue can only be given as between 0.28 and 0.34 in.

The data for maximum drainage rates are summarized in Table 3. The first column under each date shows the maximum rates; these fall off with increasing depth in the same way for each date. The second

column gives a measure of the resistance to water movement; this is corrected for viscosity differences in the third column. This correction assumes that the viscosities at all depths are determined by the mean soil temperature (at 4, 8 and 12 in.) on the morning of the rainfall. For the shallowest gauge this is probably of the correct order, but may be considerably in error for the others. For instance; if the gauges were not partly isolated from heat movements from below one would expect the June and October temperatures to be nearly equal at 40 in., i.e. the best comparison between June and October for the deeper gauges is between the entries in column two. In the same way the February resistances are probably under-estimated. The fourth columns give the resistances of the lower two 20 in. of soil and in all three cases these are nearly equal and about five times as great as those of the top 20 in.

Table 3. *Seasonal change in maximum drainage rate*

June 1936				Feb. 1937				Oct. 1935			
Depth of gauge in.	Max. drainage rate in. per hr.	Depth \div max. drain. rate = res.	Res. \div viscosity (June $\eta = 1$)	Max. drainage rate in. per hr.	Depth \div max. drain. rate = res.	Res. \div viscosity	Δ (res./ η)	Max. drainage rate in. per hr.	Depth \div max. drain. rate = res.	Res. \div viscosity	Δ (res./ η)
20	1.00	20	20	0.66	30	20	135	0.82	25	21	87
40	0.32	125	125	0.17	235	155	127	0.32	125	108	93
60	0.25	240	240	0.14	428	282	0.26	0.26	231	201	

It appears then, that the gauges have a system of comparatively wide drainage channels which may extend down to about 20 in.; below this depth the structure is nearly uniform, the drainage channels being narrower and/or fewer. Fig. 1 and Table 3 suggest that there is a slight change in water-holding capacity between June and February. Thus from Table 3 the ratio of the February and June resistances for unit viscosity below 20 in. is 1.19, and as the resistance is proportional to the inverse cube of the channel width, the change in the latter is about 6 %. The corresponding value for the February-October change is about 15 %. The changes in the air content at field capacity will thus be of the same order and the next section will show that these changes in a small quantity are quite insignificant fractions of the water content at field capacity.

The air content of the drained soil.

From Fig. 2 it is quite clear that starting from a waterlogged state, but with no water lying on the surface and no more rain falling, the 20 in.

gauge would only discharge about 0.32 in. When the soil is waterlogged practically all the air is driven from its pore space. Hence when drainage stops and no water has been lost by evaporation only $0.32 \times 100/20 = 1.6\%$ of the volume of the 20 in. soil block is occupied by air. Although this may seem a small value for the air content of fully drained soil, it is quite consistent with the volume, weight and water content of a series of soil samples taken from fallow land close to the gauges in 1870.

The sampling tool was an iron frame 9 in. deep and 6 in. square. It was driven in till its upper edge was flush with the surface of the ground, and the soil so enclosed was dug out to 9 in. and immediately weighed. The surrounding soil was then removed, the frame was driven down another 9 in. and a second sample was dug out and weighed. The process was repeated till six samples had been taken. The samples were oven dried and reweighed in the laboratory.

The intention when the samples were taken was to find the moisture content of the soil, and only the percentages of water in the samples (excluding stones $> \frac{1}{4}$ in.) were published at the time (Lawes & Gilbert, 1871). The experimental figures have been preserved and are given in

Table 4. *Samples taken from fallow soil close to the drain gauge in 1870*

9 in. section	Sample as taken (stones included)	Sample oven dried (stones included)	Water by difference oz.	Vol. of solids ($\rho = 2.7$) fluid oz.	Vol. of solids plus water fluid oz.	Nominal vol. of sample fluid oz.
1	339	291	48	112*	160	187.5
2	339	251	88	93	181	187.5
3	341	238	103	88	191	187.5
4	348	252	96	93	189	187.5
5	354	256	98	95	193	187.5
6	328	236	92	88	180	187.5

* Specific gravity of solids in first section taken as 2.6.

Table 4. The second column shows the original wet weight (stones included), the third the dry weight (stones included) and the fourth the water content of the samples in ounces. Assuming that the solids have a mean specific gravity of 2.7, the fifth column gives the volumes occupied by the solids in fluid ounces. The sixth column gives the volume occupied by solids and water in fluid ounces.

The volume of each sample should have been $3/16 = 0.1875$ cu. ft. = 187.5 fluid oz. It will be seen that in three cases the combined volume of solids and water exceeds 187.5 fluid oz. Owing to the difficulty of driving the frame down precisely 9 in. each time, the total volume of each

sample evidently was not exactly 187.5 fluid oz. Consequently we cannot compute the air content precisely; but it is evident that below 9 in. it is quite small. The higher air content of the top 9 in. must be attributed partly to loss of water by evaporation and partly due to loose structure produced by cultivation. The top soil of the drain gauges has not been cultivated since 1870, and the estimated air contents of 1.6% in the top 20 in. of soil and of 0.7% in the top 60 in. (i.e. 0.25% in bottom 40 in. of deepest gauge) are not inconsistent with the sampling figures.

B. Drainage during rain

The first response of the gauges occurs some time after the onset of rain, the delay being ascribable to two causes. The first is the need to bring the soil moisture up to field capacity; if the quantity of rain falling

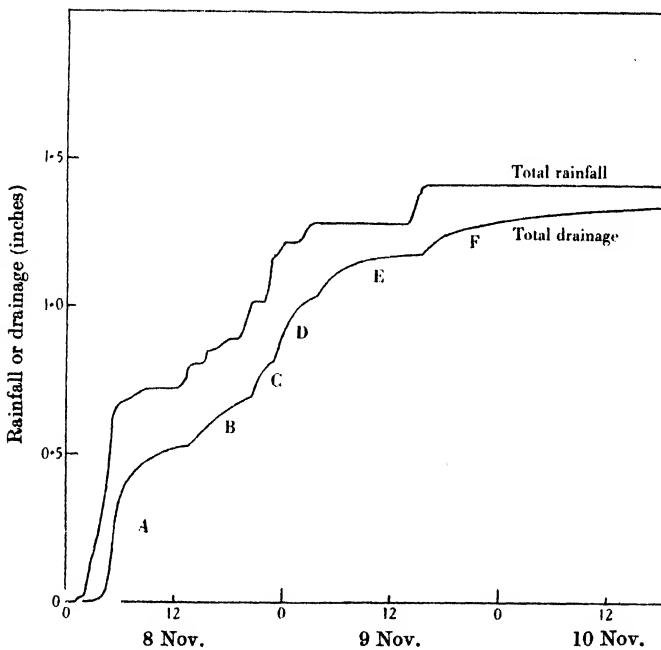


Fig. 3. 20 in. drainage curve (8-10 Nov. 1926).

is not sufficient to do this, then, with occasional exceptions to be noted below, there will be no drainage. The second is due to the finite time required for the water to move down from the surface to contribute to the head causing drainage. Assuming that the rate of rainfall is steady,

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the drainage head increases steadily, and with it the drainage rate, until the latter is equal to the rainfall rate. A change in rainfall rate tends to produce a corresponding change in drainage rate but because of the time lag in moving through the gauge the adjustment to new conditions is not instantaneous and the drainage curve thus tends to give a smoothed out picture of the rainfall curve at an earlier epoch. The curves for November 1926 (Fig. 3) show this and also the time lag in the response to new falls

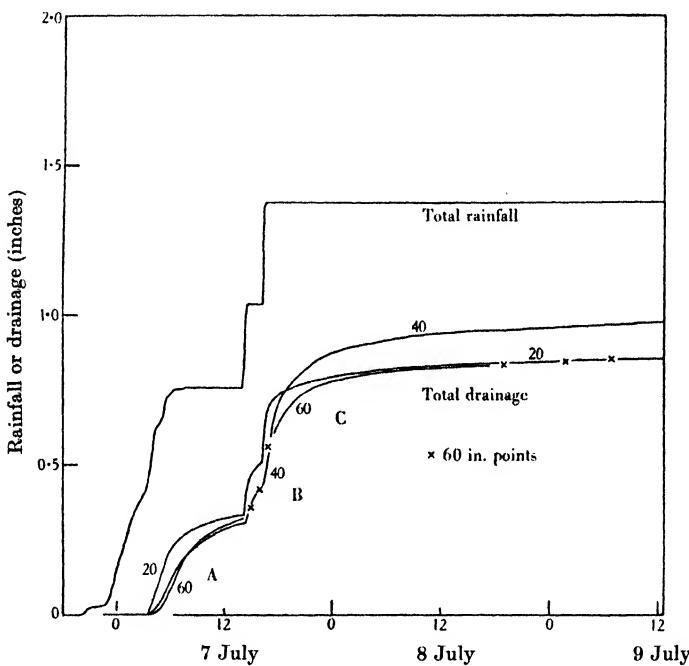


Fig. 4. 20, 40 and 60 in. drainage curves (6-9 July 1927).

of rain. The effect of previous evaporation on the response is shown in the lower part of Fig. 4, where about 0.45 in. of rain fell before drainage began. The total rain in the first fall was 0.76 in. and from the 30° point and Table 1 the drainage would have been 0.35 in., i.e. 0.41 in. were needed to bring the soil moisture up to field capacity. This is less than the amount which had fallen when drainage began, the difference being a measure of the lag in response of the gauge, and this is the general rule for the behaviour of the gauge. There are exceptions, one of which is illustrated in Fig. 5, imperfectly because of the reduced scale, and details quoted below are taken from the 20 in. charts. The rain on 13 August

1937 ended a warm dry period of 3 weeks. In the first shower 0.17 in. fell without effecting drainage; when the second heavy fall occurred 8 hr. later, most of the first would probably have re-evaporated; because of this uncertainty, two figures will be given for rainfall: R and $(R + 0.17)$. The drainage response to the two later showers gave curves which were identical beyond the 30° point and the second was used to estimate the drainage residue of the first. Drainage began at 5.45. Previous rain was

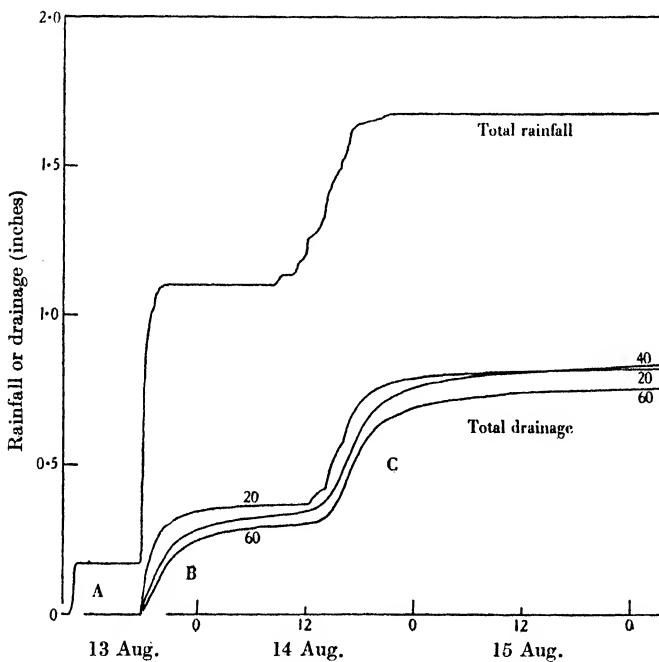


Fig. 5. 20, 40 and 60 in. drainage curves (13-15 Aug. 1937).

0.105 (0.275) in. The total drainage was 0.385 in., the total rain was 0.935 (1.105) in., i.e. the deficit was 0.550 (0.720) in. If the rain had fallen slowly, gradually bringing up the gauge to field capacity, an amount at least equal to the deficit would have been needed to start drainage; the observed value is very much less, suggesting that water passed through the gauge without first saturating the soil. Part of this would pass down the gap between the soil and its retaining wall, and part through the body of the soil, where, because of the very rapid rainfall rate, air in the pores would be unable to escape, thus making these pores inaccessible to water which would then appear as abnormal drainage. The effect is

one which is to be anticipated whenever rain starts and is maintained at a rapid rate of fall on soil which contains air. Returning to Fig. 4, we have a further example in the response to the later rains on 7 July. The first of these two heavy showers occurred after air had had an opportunity of entering the soil; it amounted to 0.28 in. and was followed about 2 hr. later by a fall of 0.34 in. We have estimated the drainage responses (from 30° points) as 0.24 in. for each fall, i.e. the deficits are 0.04 and 0.10 in. respectively. The evaporation opportunities for producing these deficits were 7 hr. mostly sunny, about midday, and 2 hr. about midnight; hence one would anticipate a comparatively large deficit for the first and a very small one, probably zero, for the second. We conclude that air was entrapped during the earlier of these two heavy falls; there is no evidence to show that the same was not true of the second.

There may be abnormal drainage in winter. During falls of snow the gauges may receive more or less than the average amount because of drifting. If the ground is frozen before snow falls, the eventual drainage will depend upon the rate at which the soil thaws as well as that at which the snow melts. Snow fell in January 1940 after a long period of frost in which the soil froze down to a foot or more. During the eventual thaw the 60 in. gauge was first to begin running, then 40 in. a day later and finally 20 in., a further day later, suggesting that the shallowest gauge had been frozen from the bottom as well as from the top. Even without snow, the effect of frost is important. It has a drying action and in addition to immobilizing water which would otherwise drain away, it empties some of the smaller pores which would not normally be emptied by free drainage. Thus frost during a drainage period will hold water back and whilst the ice is still present further rain will have this extra deficit to make up. When the ice thaws, the amount frozen will, of course, be added on to the normal drainage. The first and third of these phases can be seen in Fig. 3. The grass minimum on 8 November was 26° F.; on the 9th, 35° F. The deficit of the first shower is obviously greater than the final deficit and drainage was presumably held up by freezing, the ice melting slowly until the penultimate shower of 9 November, when the deficit became appreciably less.

The information from the 20 in. curves of Figs. 3-5 is summarized in Table 5.

Between periods B and C of November 1926 there is a slight increase in the accumulated deficit which may be due to the drying action of the frost, but as the magnitude of the change is close to the inaccuracy of extrapolation, the evidence is not conclusive.

Table 5. *Effect of soil conditions on drainage*

Date	Drainage period	Rain	Estimated drainage	Rain when drain ran	Deficit	Accumulated deficit
Nov. 1926	A	0.72	0.60	0.17	0.12	0.12
	B	0.17	0.18	0.07	-0.01	0.11
	C	0.125	0.10	0.12	0.025	0.135
	D	0.205	0.21	0.15	-0.005	0.130
	E	0.060	0.11	0.06	-0.05	0.080
	F	0.130	0.15	0.12	-0.02	0.060
July 1927	A	0.76	0.35	0.45	0.41	0.41
	B	0.28	0.24	?	0.04	0.45
	C	0.34	0.24	?	0.10	0.55
Aug. 1937	A	0.17	0.00	—	0.17 +	0.17
	B	0.93	0.38	0.10	0.55	0.72
	C	0.58	0.44	0.16	0.14	0.86

* Uncertain because of stretching of recording paper in humid atmosphere; this is unimportant in November 1926, and allowed for in all charts after 1934 and thus in August 1937.

C. Natural periods

The small and constant values of the monthly drainage residues (Table 1), obtained for widely differing drying conditions at the surface, suggest that when evaporation begins at the surface the moisture distribution in the gauge is such that upward movement is confined to the surface inch or two and drying at the surface during the period of drainage does not affect the total discharge. Assuming that, in general, drainage only occurs when the water content exceeds field capacity, the moisture changes between the ends of two falls of rain causing drainage can be written

$$(FC + DR_1) - DR_1 - E_{12} + R_2 = FC + (D_2 - DR_2) + DR_2.$$

Otherwise: at the time the rain stops the gauge contains its field capacity (FC) and a drainage residue (DR_1). Losses of two kinds occur: (1) the drainage residue is gradually discharged, and (2) evaporation (E_{12}) occurs from the surface. Rain (R_2) falls, and after bringing the gauge to field capacity, a certain amount drains during the rain period ($D_2 - DR_2$), leaving, at the end of the fall, a residue DR_2 , which must of course make the total drainage equal to D_2 . The equation reduces to

$$E_{12} = R_2 - D_2.$$

If R_2 falls in one shower, E_{12} is the total amount lost by evaporation. If between R_1 and R_2 there is a number of small falls which do not produce any drainage, then E_{12} is the net loss by evaporation: the total evaporation over the period is

$$\Sigma E_{12} = \Sigma R - D_2,$$

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where ΣR includes R_2 . Obviously we may add the evaporation for a number of such periods to obtain

$$\Sigma E = \Sigma R - \Sigma D, \text{ i.e. evaporation} = \text{deficit (p. 1),}$$

where the following conditions must be noted:

(1) ΣR is measured from the end of one fall of rain causing drainage to the end of another also causing drainage.

(2) ΣD is measured between the cessations of the drainage responses to these falls of rain, i.e. *the drainage period is not in phase with the rainfall period.*

Periods which satisfy these conditions we shall call "natural periods". They may be as short as a few days; they may extend over several months. For certain arbitrary periods the error in assuming that they are natural periods is small. Thus for a year (1 January–31 December) the phase difference is unimportant and as December and January are normally months of heavy rainfall, the showers of which rarely fail to cause drainage, the evaporation attributed to 365 (6) days ($\Sigma R_y - \Sigma D_y$) may actually be due to a few days more or less—a negligible error. Similarly, periods of six calendar months which begin and end in seasons of comparable weather may be treated as natural periods. Thus in the later general discussion we shall consider April to September, and October to March as approximating closely to natural periods, but one unsatisfactory aspect of a division between September and October will shortly appear. Individual calendar months will rarely be natural periods. The phase difference may be important in cases where rain falls on the last day of the month; in winter months, for instance, neglecting it may lead to an estimate of drainage which exceeds the month's rainfall. More generally, the beginning of the natural period, which is the end of the last fall of rain causing drainage preceding the beginning of the month, may be several days, or in a dry summer perhaps several weeks earlier than the first day of the calendar month, so, assuming that the end of the natural period is the end of the calendar month, the deficit ascribed to the calendar month will actually be due to the evaporation over a much longer period. Similarly, assuming the beginning of the calendar month to be the beginning of a natural period, the latter may only last for a few days, the rest of the month being rainless, or, less seriously, drainless. In this case the estimated deficit for the month will be due to evaporation over a shorter period.

Another trouble arises when the deficits are correlated with meteorological conditions. For instance; an October deficit may be due to a

period including two weeks of September. Ascribed to the 31 days of October it will be an over-estimate and the correlation will be further vitiated by the assumption that the deficit is due entirely to the meteorological conditions during these 31 days.

Over a long period of years, and where there are no violent changes of weather from one month to another, it is probable that errors will cancel out. Thus the aggregate deficit for 50 Augments probably corresponds to the evaporation of 50 months having the mean meteorological conditions of 50 Augments. October is a likely exception. September is comparatively warm and dry; October is colder and is the wettest month of the year. It is very probable that the October deficit often includes a contribution from dry days at the end of September which is not compensated by a corresponding loss from October to November. Thus for October we may expect

$$\Sigma R_0 - \Sigma D_0 > \Sigma E_0.$$

These approximations to natural periods will be used in the later general discussion and also in a short note by Mr Sahni which follows this account. He considers periods of 2-13 days, using the daily totals of rainfall and drainage. In practice one cannot always be certain of the last rain to cause drainage; a heavy fall causing drainage may be followed 12 or 24 hr. later by a light shower whose effect on the drainage cannot be established with certainty. Thus for a rapid survey from the daily totals it is more convenient to take the period as between the last days of drainage, or between very low drainage minima (< 0.005 in. per day). The error may be of the order of one-half to one day, i.e. of the same order as that which would be present in any case in using daily totals. From this it would seem that no period shorter than 5 days should be considered, but as Sahni's data will show, it is possible to work with 2-day periods which, with one exception, show no anomalous behaviour.

DAILY TOTALS OF RAINFALL AND DRAINAGE

Drying out of 20 in. gauge

In the preceding section we saw that the deficit for a given fall is a measure of the net water lost by evaporation in a previous period of drying. From earlier sections and Figs. 3-5 it will be apparent that, except for rainfalls near the end of a meteorological day, the greater part of the drainage is complete on the day of the rain, and to a very good

approximation the difference between the daily totals may be taken as giving the deficit.

The values of the deficit so obtained will be over-estimates of the evaporation, but they can be very quickly read off from the daily totals to give the order of magnitude of the water loss from fallow soil during dry seasons. Table 6 (a) shows all the occasions between 1925 and June 1936 when this water loss exceeded about 0.40 in. The second part (b) of the table is a more detailed record of the responses to heavy rain in all seasons for 1936-9 in which the values of D have been estimated from the charts, and the amounts of rain required to start drainage have been included. Comparing these with the values of $R - D$, they appear to be generally of the same order. There are a few cases in which the rain needed to start drainage is appreciably less than $R - D$ and these probably arise from the entrapping of air by rapidly falling rain.

The first part of the table shows that in normal summers the net water loss rarely exceeds 0.7 in., this being the amount to bring the soil to field capacity. We have already seen that to waterlog the soil about 0.3 in. of water is needed in excess of field capacity (p. 82) so that in the dried-out state of a normal summer only 1 in. of water would be needed to replace the total air content of the soil of the 20 in. gauge, i.e. the air content of this fallow undisturbed soil rarely exceeds 5 % of the total volume. The effect of cultivation (again without crop) appears

Table 6. *Response of 20 in. gauge to rain*

(a) 1925-36

Date	R	D_{20}	$R - D_{20}$	Date	R	D_{20}	$R - D_{20}$
20. vii. 25	0.85	—	0.85	2. iv. 31	0.44	—	0.44
22. vii. 25	1.10	0.67	0.43	5. vi. 31	0.41	—	0.41
23. viii. 25	1.37	0.82	0.45	14. vii. 31	1.18	0.47	0.71
2. vi. 26	0.64	0.23	0.41	2. ix. 31	0.47	0.07	0.40
4. vii. 26	0.45	—	0.45	21. v. 32	0.72	0.30	0.42
19. vii. 26	0.45	0.02	0.43	30. vi. 32	0.68	—	0.68
18. vi. 27	0.39	—	0.39	11. vii. 32	0.49	0.01	0.48
22. vi. 27	0.46	—	0.46	11. viii. 32	0.69	0.08	0.61
6. vii. 27	0.77	0.30	0.47	20. viii. 32	0.69	0.04	0.65
11. vii. 27	0.52	0.07	0.45	22. ix. 32	0.50	0.09	0.41
7. viii. 27	0.70	0.08	0.62	12. ix. 33	0.53	—	0.53
9. ix. 27	0.78	0.16	0.62	25. vi. 34	0.76	—	0.76
27. vii. 28	0.84	0.10	0.74	28. viii. 34	0.75	—	0.75
12. iv. 29	0.43	0.02	0.41	15. ix. 34	0.98	0.32	0.66
24. v. 29	1.04	0.40	0.64	18. v. 35	0.42	—	0.42
1. x. 29	0.68	—	0.68	30. v. 35	0.42	—	0.42
5. x. 29	0.67	0.01	0.66	18. vii. 35	0.64	—	0.64
20. x. 29	0.72	0.32	0.40	30. viii. 35	0.64	—	0.64
3. iv. 30	0.50	0.04	0.46	12. vi. 36	0.48	—	0.48
25. v. 30	0.51	0.13	0.38	21. vi. 36	3.20?	2.62?	0.58?
18. vi. 30	0.72	0.06	0.66				
20. viii. 30	0.42	—	0.42				
13. ix. 30	0.40	0.01	0.39				

Table 6 (cont.)

(b) 1936-9

Date	R	D	R - D	Rain when drain ran	Previous rain
1936					
2. vi.	0.48	—	0.48	—	0.53 in preceding month
12. vi. •	{ 0.54 0.33	— 0.15	0.54 0.18	— 0.15	0.80 in preceding 12 days
21. vi.	{ 0.92? 1.87?	?	?	0.47 0.12	0.61 in preceding 7 days
2. vii.	0.49	0.22	0.27	>0.25	0.66 in preceding 7 days
7. vii.	0.75	0.34	0.41	0.24	0.49 5 days before
10. vii.	0.47	0.15	0.32	0.31	0.75 3 days before
15. vii.	0.70	0.43	0.27	0.39	0.33 2 days before
20. ix.	1.18	0.75	0.43	0.42	{ 0.18 in preceding week 1.50 in preceding 3 weeks
31. x.	0.64	0.54	0.10	0.18	0.95 in preceding 3 weeks
11. xi.	0.96 + ?	?	?	0.21	1.13 in preceding 10 days
1937					
16. iv.	1.01	0.90	0.11	0.22	0.92 in preceding 10 days
11. v.	0.68	0.39	0.29	0.16	0.14 5 days before
20. v.	0.59	0.32	0.27	0.36	0.18 2 days before
23. v.	0.50	0.35	0.15	0.30	0.50 3 days before
13. viii.	{ 0.26 0.94	— 0.36	0.26 0.58	— 0.08	0.00 in preceding 3 weeks
14. viii.	0.58	0.45	0.13	0.15	1.20 on previous day
15. ix.	0.49	0.01	0.48	0.15	0.45 in preceding 4 weeks
17. ix.	{ 0.33 0.46	0.21 0.46	0.12 0.00	0.08	0.26 on previous day
1938					
15. i.	0.49	0.49	0.00	0.08	0.25 on previous day
12. viii.	0.79	0.25	0.54	0.56	0.68 in preceding week
28. viii.	0.45	—	0.45	—	0.43 in preceding 16 days
18. xi.	0.45	0.30	0.15	0.20	0.34 in preceding week
25. xi.	0.63	0.58	0.05	0.16	0.99 in preceding week
27. xi.	0.40	0.38	0.02	0.14	0.63 2 days before
9. xii.	0.82	0.71 app.	0.11 app.	0.24	0.20 in preceding 5 days
1939					
29. iv.-1. v.	1.55	0.92	0.63	0.56	0.47 in preceding 3 weeks
15. v.	0.57	0.24	0.33	0.35	0.33 on previous day (no D)
10. vi.	0.55	—	0.55	—	0.00 in preceding 3 weeks
15-16. vi.	0.30 + 0.23	0.00 + 0.12	0.41	0.53	0.37 in preceding 4 days
2-3. viii.	0.40 + 0.39	0.00 + 0.27	0.52	0.55	0.35 in preceding 11 days
20-21. viii.	0.24 + 0.40	0.00 + 0.17	0.47	0.56	0.00 in preceding 9 days
1. ix.	0.55	0.11	0.44	0.52	0.00 in preceding 5 days
2. ix.	0.32	0.22	0.10	0.29	0.55 on previous day
8-9. x.	0.50 + 0.24	0.39	0.35	0.39	0.40 4 days previous (no D)
13-15. x.	1.69	1.46	0.23	0.21	0.06 in preceding 3 days
22-27. xi.	1.69	1.57	0.12	0.12	0.01 in preceding 3 days

in the data for the top 9 in. for midsummer 1870 (Table 4). The air content is about 15 % in this layer, and 8 % for the top 20 in., showing an appreciable increase.

The second part suggests that the greater part of the loss occurs in the first few days after rain. Thus the deficits after 5 days were 0.4 and

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0.45 in. (7 July 1936 and 11 July 1927); after 9 days, 0.65 in. (20 August 1932); and after 3 weeks' drought, only 0.84 in. (13 August 1937). An extreme case occurred in 1921, when after a severe drought interrupted by nominal rainfall in June, July and August, a fall of 2.0 in. was recorded early in September, the responses being:

$$\begin{aligned} D_{20} &= 0.70, & R - D_{20} &= 1.30, \\ D_{40} &= 0.63, & R - D_{40} &= 1.37, \\ D_{60} &= 0.59, & R - D_{60} &= 1.41. \end{aligned}$$

Incidentally the response of the gauges is in order of depth. The log book of the gauges records the presence of cracks in the soil at the beginning of the fall, and the first part of the drainage would certainly be expedited by these cracks, but by the end of the drainage period the soil would be saturated and we may take the above figures as a measure of the extent of the drying out under extreme conditions. These examples illustrate what is apparent from the complete records, that the initial rate of drying is high (while the surface is wet) but becomes very low when the top layer of soil has dried. We should thus expect more evaporation when a given amount of rain is spread over a number of showers, especially in summer.

Effect of rainfall distribution on drainage

In Table 7 are shown the effects of a difference in rainfall distribution in summer and in winter. These are taken from the daily totals and show (a) the response to heavy falls concentrated in one day, and (b) similar total falls spread over a month, (i) in winter, and (ii) in summer. Heavy falls in a short time produce an appreciable amount of drainage, whatever the season, but if the same amount falls in a series of small isolated showers there may be no drainage during summer months whereas the winter drainage is not appreciably reduced.

We may, therefore, expect a regression equation connecting drainage and rainfall to be more accurate for winter months than for summer months.

Table 7. *Effect of distribution on response of 20 in. gauge*

(a) Heavy falls (>1.00 in.) in a day

Date	R	D
22 July 1925	1.100	0.673
23 Aug. 1925	1.370	0.822
28 Jan. 1927	1.070	0.896
14 Sept. 1927	1.703	1.516
28-29 Nov. 1927	1.318	1.288
24 May 1929	1.040	0.405
14 July 1931	1.182	0.468
21 June 1936	3.200?	2.618?

Table 7 (cont.)

(b) Similar falls spread over a month

(i) Winter		(ii) Summer		
Month	R	D	Month	
Feb. 1929	0.789	0.708	June 1929	1.023
Feb. 1930	0.855	0.612	July 1929	1.417
Mar. 1930	1.451	0.712	June 1931	1.520
Jan. 1931	1.704	1.231	June 1932	0.850
Dec. 1931	1.109	0.643	June 1933	1.033
Dec. 1932	0.733	0.453	July 1933	1.425
Nov. 1933	1.471	0.890	June 1934	1.750
Jan. 1935	1.072	0.692	July 1934	1.130
Mar. 1936	1.413	0.437	July 1935	0.961
			Aug. 1935	1.635
			July 1937	0.000
				0.779

Comparison of the gauges

If the three gauges differed only in depth one would expect certain regular differences in the responses to rain. These would be most marked in periods of intensive drying when the gauges might be expected to respond in the order $D_{20} > D_{40} > D_{60}$. The annual totals show that D_{40} has, with few exceptions, been greater than either D_{20} or D_{60} , the difference between these being small and of variable sign. Various explanations of this departure from expectation have been offered, including differential washing down of silt, differences in physical structure, and a leak in the 40 in. gauge casing which Lawes & Gilbert re-cemented in 1874.

Examination of the 1921 drought data (above) shows that the responses were in the expected order. This is usually true after dry periods when the automatic charts indicate that the first response is $D_{20} > D_{40} > D_{60}$. After a further fall the order gradually changes to $D_{40} > D_{20} > D_{60}$, and still more rain leads to $D_{40} > D_{60} > D_{20}$ (see Figs. 4, 5). This suggests that there is a leakage through the cement casing of the 40 in. gauge, and perhaps of the 60 in. gauge also, that occurs when the outer soil is very wet. Confirmation of this suggestion has been made in two ways.

Miller gives figures for the chloride content of rain and drainage water for the period 1877-1904. The annual means are:

Rain	15.15 lb. per acre,
20 in. gauge:	14.84 lb. per acre,
40 in. gauge:	15.89 lb. per acre,
60 in. gauge:	14.64 lb. per acre.

The corresponding data obtained by Miller up to 1915 (reviewed by Russell & Richards 1920) show similar agreements and differences and

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it is obvious that more chloride is coming through the 40 in. gauge than is falling on the surface in the rain and we must conclude that the extra amount is being drawn from soil outside the gauge, i.e. there is a leak into the 40 in. gauge. The symptoms are compatible with a crack fairly near the surface which is only effective when rainfall has been sufficient to saturate the grass-covered surround, i.e. when the fall has made up the deficit due to combined evaporation and transpiration of the grass.

A direct test was made in June 1939, when, after a dry May, the ground round the gauges had shrunk away from the walls. Water was run into the fissures and after 18 hr. the gauges recorded:

$$D_{20} \ 0.000; \ D_{40} \ 0.020; \ D_{60} \ 0.007.$$

Both chloride and direct test lead to the conclusion that the 40 in. records have been affected by leakage. The evidence is not so conclusive for the 60 in. gauge. Comparison of the monthly values of D_{20} and D_{60} , shows that the former is usually greater in autumn and early winter, but from January into the spring the reverse is the case. The first effect is presumably due to the deeper drying but this is masked in the wet winter months by slight leakage. After dry summers D_{20} may be persistently greater than D_{60} for every month from September to the following February (e.g. 1914, 1921) whereas after wet summers the change-over occurs in October-November. These effects can be partly accounted for by a leak in the deeper gauge, and while the existence of a leak is non-proven, the fact that there is reasonable doubt about the water tightness of the walls is held to be sufficient reason for leaving consideration of the 60 in. records out of the later general discussion.

PUSA DRAIN-GAUGE DATA

At this stage it will be useful to consider how a difference in soil and weather conditions affects the drainage response. Leather (1911) gives daily rainfall and drainage totals for gauges at Pusa. The drain gauges are 1/1000 acre in area, two being 6 ft. deep and two 3 ft., one in each pair being cropped and the other left fallow. The soil is alluvial with 40 % of fine chalk, the first 2 ft. being loam, the next two more sandy and the bottom 2 ft. are stiff clay. Run-off is measured when standing water would exceed 2 in. The data cover the years 1906-10 but our discussion will be confined chiefly to those for 1909 in which rain was heavy and drainage considerable. Leather's main conclusion was that water passed very uniformly through the soil and not chiefly by means

of large channels. The daily records indicate that the gauges run for 5 or 6 days after rain, about twice as long as the Rothamsted gauges. From the nature of the soil, one would expect the Pusa time to be shorter, assuming equal degrees of homogeneity, and the fact that the Rothamsted gauges discharge completely in a shorter time seems to indicate that they have some coarse macrostructure which facilitates percolation. The Pusa records show that where root holes are present they assist drainage. (a) The cropped gauges respond to rain about one-half to one day earlier than the fallow gauges. (b) The maximum daily amounts of drainage are larger for the cropped than for the fallow gauges. On three occasions the cropped gauges exceeded 3 in. per day, and exceeded 2 in. seven times. The fallow gauges never exceeded 3 in. in a day and only twice exceeded 2 in. (c) Where run-off has been recorded the amounts are greater from the fallow gauges. Summarizing, the cropped gauges appear to be more permeable so that (a) they respond more rapidly to rain, (b) larger amounts can pass through quickly, and (c) surface accumulation is less.

An estimate of the extent to which the soil had dried cannot be made as precisely as was possible with the Rothamsted data because of the longer time lag between the incidence of rain and onset of drainage, the longer period for which the drains run, and the high rate of evaporation while the surface is wet. Making allowances for these factors so that the figures for the drying out is an over-estimate, it appears that at the beginning of the 1909 monsoon, after 21 months without drainage, it was about $12\frac{1}{2}$ in. for the deep gauges and 10 in. for the shallow ones. The drying out before the monsoon of the following year was much less, being about 3 or 4 in. This is probably a more normal value than the other, because the effect of 21 months drying had probably extended so far down the gauge that convection of air through the soil could take place easily, and the drying rate would be accelerated thereby. This type of drying would not occur in a field soil, and it is possible that 10 in. is a considerable over-estimate of the loss from field soils even in abnormal years.

GENERAL DISCUSSION OF SEASONAL EVAPORATION

This discussion is primarily an application of the conception of natural periods to the detailed analysis of Koshal. He obtained partial regression equations representing the drainage to be expected in any month in terms of the mean air temperature and rainfall of the month, with allowance for secular changes in these variables. Postponing considera-

tion of the secular changes, we have to consider a series of equations of which the following, for January, is typical:

$$D_{20} = 1.9043 + (0.96729 \pm 0.03848) R' + (0.00232 \pm 0.01294) T',$$

where R' and T' are deviations from the monthly means. The equations can be rearranged to give expressions for the deficit as in Table 8.

Table 8. *Monthly values of deficit ($R - D_{20}$) (from Koshal)*

Jan.	$0.398 + (0.033 \pm 0.038) R' - (0.0023 \pm 0.0129) T'$
Feb.	$0.463 - (0.040 \pm 0.037) R' + (0.0336 - 0.0136) T'$
Mar.	$0.893 + (0.072 \pm 0.056) R' + (0.0538 \pm 0.0242) T'$
April	$1.365 + (0.323 \pm 0.050) R' + (0.0134 \pm 0.0265) T'$
May	$1.578 + (0.389 \pm 0.041) R' - (0.0633 \pm 0.0228) T'$
June	$1.627 + (0.455 \pm 0.058) R' + (0.0204 \pm 0.0419) T'$
July	$1.965 + (0.504 \pm 0.040) R' - (0.0071 \pm 0.0244) T'$
Aug.	$1.936 + (0.306 \pm 0.049) R' - (0.0200 \pm 0.0341) T'$
Sept.	$1.485 + (0.271 \pm 0.037) R' - (0.0583 \pm 0.0263) T'$
Oct.	$1.331 + (0.123 \pm 0.031) R' + (0.0006 \pm 0.0198) T'$
Nov.	$0.652 + (0.029 \pm 0.028) R' - (0.0147 \pm 0.0137) T'$
Dec.	$0.402 + (0.015 \pm 0.027) R' - (0.0170 \pm 0.0140) T'$

The non-significant regression coefficients are given in italics; those which are significant are in ordinary type, and it will be seen that the temperature terms are generally non-significant and not all of the same sign. The rainfall terms fall into two groups. Five winter months have non-significant coefficients, and if to these we add October as having the smallest coefficient of the remainder, the year can be divided into two equal periods for which we may equate the deficit and evaporation with some confidence. Let us compare the parts with the whole.

Annual evaporation

If all the monthly expressions are taken together to give a value of the deficit for the whole year we obtain, neglecting standard errors, an equation containing only R' :

$$E_{an.} = R - D = 14.10 + 0.20R',$$

where R' is now the deviation from the mean *annual* rainfall. This equation summarizes Miller's observations, the difference between annual rainfall and annual drainage being very nearly constant, but tending to increase with annual rainfall. Fig. 6 shows the data for the period covered by Koshal's analysis. There is a considerable scatter of the points, but the positive regression coefficient is apparent. The data for the two halves of the period have been kept distinct and there is evidence of a secular change in the deficit. The values for the earlier

period tend to be greater than those for the later period, indicating that the deficit has tended to decrease with time, or, from the view point of Russell and Koshal, annual drainage has tended to increase with time.

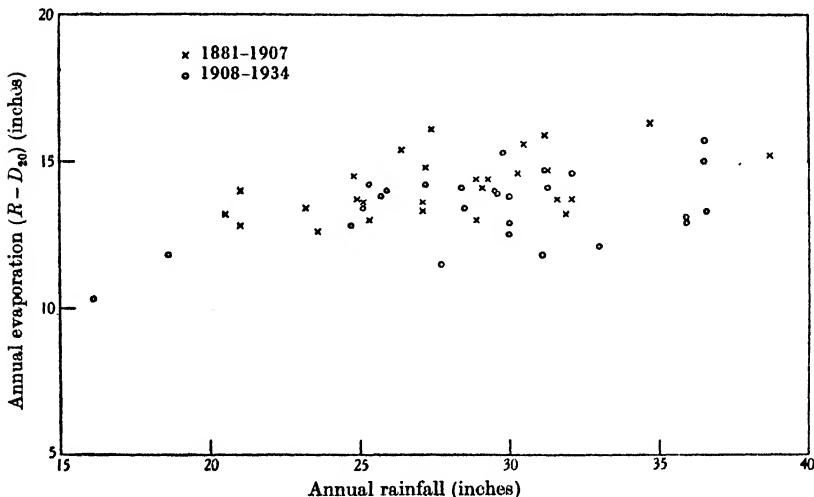


Fig. 6. Annual evaporation from fallow soil (Rothamsted).

Summer and winter evaporation

The data for the 6-month periods are shown in Fig. 7. The points for the two parts of the 50-year period are again distinguished and the figure gives a general picture of the difference in the influence on evaporation of summer and winter weather. A line of unit slope has been included ($E = R - D = R$) which represents arid conditions in which there is no drainage. No points can be above this line. The figure shows several interesting points:

- (1) As one would expect, evaporation is greater in summer than in winter.
- (2) There is a positive regression on rainfall for the summer months and a non-linear regression equation would be needed to fit the data.
- (3) The winter points show the anticipated independence of rainfall.
- (4) The summer scatter is much less than that for winter and it is probable that most of the summer scatter is due to distribution of rainfall. Rainfall distribution in winter is of little importance and other reasons for the winter scatter must be sought (p. 92 above).
- (5) There is no evidence of secular change in either winter or summer data; the points for the two 25-year periods are well mixed, parallel

to the $R - D$ axis; there is, however, bias toward heavier winter falls and lighter summer falls in recent years.

In an analysis of the Rothamsted rainfall data for 1854-1929, Wishart (1930) found that the winter rainfall (November-April) had increased and that of other months had diminished. As Fig. 7 shows, most of the winter rain appears as drainage, and if an increasing fraction of the annual rainfall occurs in winter, the annual drainage will increase. Thus the secular change in annual evaporation may be entirely due to the secular change in rainfall distribution. It provides no clear evidence of a physical change in the gauge, though such a change may have occurred.

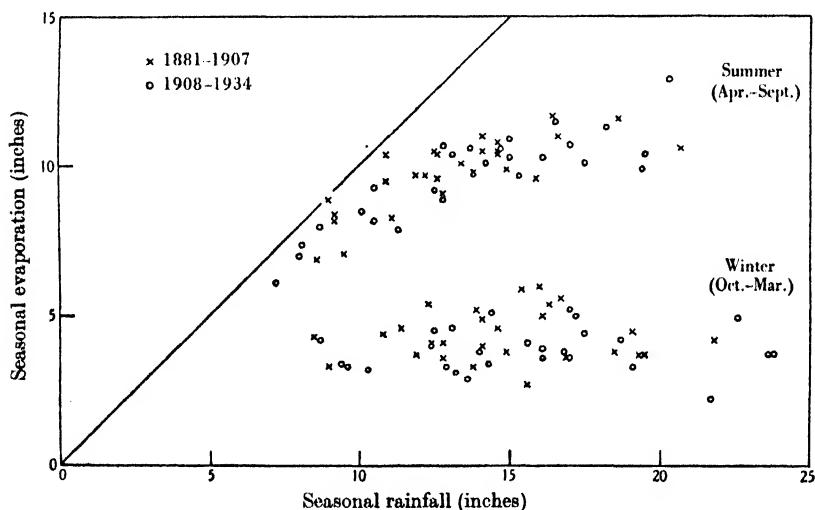


Fig. 7. Summer and winter evaporation from fallow soil (Rothamsted).

The evidence of the automatic records suggested that there is no marked change in field capacity between winter and summer. If there were such a change it might account for part or all of the scatter in the winter data, as one would expect marked differences between the deficits for winters following dry summers and for those which follow wet summers. Table 9 shows the deficits for winters following summers with (a) $\Sigma R_s < 11$ in., and (b) $\Sigma R_s > 16$ in.

The mean summer rainfall for the second group is almost twice that of the first, and remembering that the natural periods to which these are approximations will tend to include more dry days at the end of September in the first than in the second group, one would expect the

"dry summer" deficits to be slightly greater than the others. Even without this qualification, however, the difference is not statistically significant, although it is of the expected sign.

Table 9. *Winter deficits after dry and wet summers*

(a) Dry, $R_s < 11$ in.

•	Year	R_s	$(R - D)_w$	Year	R_s	$(R - D)_w$
	1887	9.194	3.720	1880	17.065	3.699
	1893	8.996	5.567	1882	16.368	4.174
	1898	9.197	4.612	1888	16.550	4.086
	1900	10.945	5.192	1889	18.642	3.560
	1901	9.480	4.407	1903	20.667	5.567
	1904	10.885	4.584	1912	17.524	4.119
	1906	8.558	5.449	1917	19.443	4.472
	1913	10.532	3.925	1918	18.216	3.323
	1914	8.741	3.694	1920	16.122	3.327
	1921	7.232	4.638	1922	16.554	3.113
	1928	10.069	4.003	1924	20.285	4.190
	1929	7.997	4.923	1927	19.517	3.783
	Means	9.33	4.557 ± 0.608		18.08	3.951 ± 0.630

Difference in mean deficit = 0.606 ± 0.875 in.

We conclude (i) that the seasonal totals show no significant effect ascribable to a change in gauge structure, and (ii) that the cause of the winter scatter must be sought elsewhere.

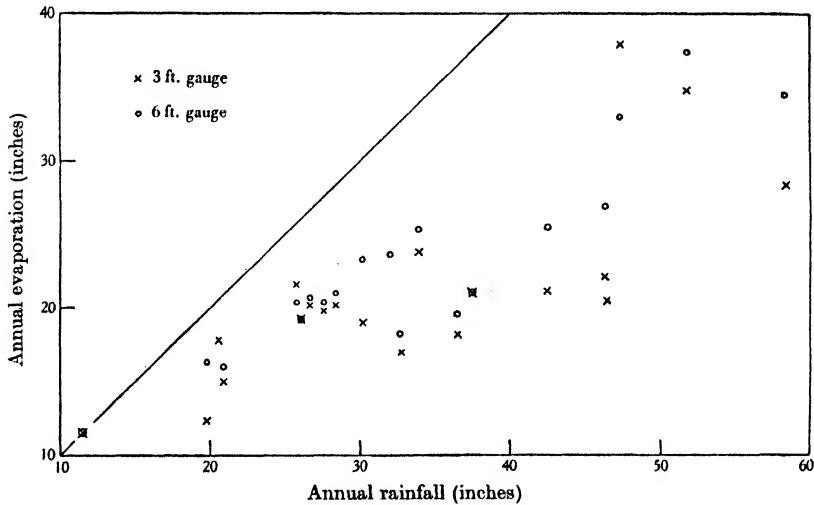


Fig. 8. Annual evaporation from fallow soil (Cawnpore).

For comparison with the Rothamsted summer data we show in Fig. 8 the annual data for Cawnpore (Leather, 1911; Batham, 1925) which should show similar features. The points are more scattered, as the rain-

fall is much more erratic in amount and distribution, but the positive regression of deficit on rainfall is apparent. On general grounds one can see why the regression line should be curved. Assuming that the rainfall is spread uniformly over the period, in very dry seasons it will all be evaporated and the points will lie on the line of unit slope. As the rainfall increases or the regularity of precipitation decreases, there will be periods in which all the rain of one fall has not been evaporated when the next fall occurs and a little will appear as drainage. As the number and intensity of the falls increases, more will be evaporated and more will come through as drainage, i.e. the curve moves away from the line of unit slope. In the limit, if there were continuous rain throughout the period, all the rain would appear as drainage, i.e. the curve would turn back and reach the *R*-axis.

Monthly values of deficit

Up to the present we have discussed evaporation during long natural periods. As shown above (p. 88) we cannot consider calendar months as such but we can discuss the modified form of Koshal's equations (p. 96) and re-examine his conclusions. The main conclusion, apart from those on secular changes and gauge differences which have already been considered, is, that because mean air temperature does not appear as a significant variable, the difference between winter and summer drainage, usually ascribed wholly to difference in evaporation, must be in part accounted for by accumulation of water in the gauges. The latter we reject because the evidence of the automatic charts and that of the effect of summer rainfall on winter deficit agree in showing that there is no significant seasonal change in gauge structure. There are two other possibilities which we shall discuss in order. The first is that the analysis was such as to conceal the effect of temperature, and the second is that mean air temperature is useless as a measure of evaporating power.

Koshal's periods were calendar months and not natural periods, and we have already outlined the nature of the errors to which this discrepancy might lead. We have also seen that in summer months, when large temperature effects are to be anticipated, that rainfall distribution has a pronounced effect on drainage, and Fig. 7 showed that the regression on rainfall in summer was not linear. These objections may not be serious, but they do raise doubts about the treatment of the temperature effect.

In any case Koshal's equations do show the effect of the increased evaporation in summer. Each month has a constant term which varies cyclically throughout the year, and these terms are the 50-year mean

values, i.e. R' and T' both equal to zero. As long period means we may consider them as arising from natural periods and see upon what factors they depend. This is the starting point of Crowther's analysis. His equation was obtained from the mean of all three gauge records so that the data were slightly different, but in effect, it was derived from the constant terms in Koshal's equations. His equation can be rearranged to read

$$R - D = 1.147 - (0.112 \pm 0.133) R_1 + (0.069 \pm 0.006) T_1,$$

where R_1 is the deviation from the mean monthly rainfall (2.44 in.), and T_1 is the deviation from the mean monthly temperature (48.1° F.). In this equation the term in R_1 is not significant, and rounding off the remainder, we have

$$E = R - D = 1.15 + 0.07 T_1$$

for any calendar month.

It is obvious that neither Koshal's nor Crowther's treatment is quite adequate by itself, always assuming that temperature is an important variable. Following this review is a note by Mr Sahni in which natural periods and a non-linear regression on rainfall are used. The results confirm those obtained by Koshal in showing no significant dependence of evaporation on mean air temperature but there is some evidence that low relative humidity and high wind velocity may facilitate evaporation.

We must, therefore, fall back upon the alternative possibility and examine in some detail how far the assumption is justified that mean air temperature is the most important single variable determining evaporating power.

FACTORS AFFECTING EVAPORATION FROM SOIL

The most striking difference between the analyses of Crowther & Koshal is that the former found a significant correlation of evaporation with mean monthly air temperature, whilst the latter's results for individual months showed little dependence on this factor. Crowther's equation expresses the common knowledge that evaporation varies cyclically throughout the year, and a significant correlation would be obtained with any other cyclic variable; Koshal's equations suggest that mean air temperature is not the best variable to choose. This can be seen if we plot mean monthly $R - D$ against T ; a fairly regular change takes place from month to month, the series of twelve points forming a loop in which the autumn values of $R - D$ are smaller than those for spring at the same mean temperature. Thus at 42° F., the monthly deficits are: 1.25 in. in spring; 0.85 in. in autumn.

Evaporation is due to a mass transfer of water vapour under a partial pressure gradient, and from soils, may be considered in three stages.

(a) *Movement in the soil.* If evaporation is to proceed steadily at the soil surface the water supply there must be replenished either by rain or by upward movement from below. The effect of rain has already been discussed and we consider the other means of supply. If, due to viscous resistance in the soil, the upward liquid movement is not sufficiently rapid, the rate of surface evaporation will decrease as the layer at 100 % R.H. retreats below the surface. Evaporation will then depend upon the resistance to the diffusion of the vapour through the air-filled pore space of the soil, and on the depth of the 100 % R.H. layer, i.e. upon the viscous resistance to liquid movement in the water-filled pore space. Prolonged drying thus sets up two kinds of resistance to evaporation, and only when the surface is kept continuously moist will evaporation be similar to that from an open water surface.

(b) *Diffusion through a still layer of air, and (c) turbulent mixing with the atmosphere.* In general there will be a layer of still air immediately above the soil (or water surface), of variable thickness, l , across which there is a vapour pressure drop, $p_s - p_a$, and the water vapour flow per unit area will be given by

$$dq/dt = D_0(T/273)^2 (p_s - p_a)/l,$$

where D_0 is the coefficient of diffusion of water vapour into air at 0° C. The value of l will depend upon wind velocity and steadiness, and the constancy of p_a will depend upon adequate mixing of the diffused vapour with the air above. It is probable that there is usually sufficient breeze to ensure that this turbulent mixing is complete, and stage (c) will not be further considered. p_a may therefore be taken as given by the air conditions several feet above the soil surface. The value of l will tend to decrease with increased wind velocity, but as it is of the order of $\frac{1}{4}$ cm. the stone coping round the gauges (10 cm. high) will act as a wind screen and we cannot predict how changes in wind velocity and direction will modify it. For the remainder of the discussion we assume that l remains constant and we have as a measure of the rate of evaporation the function, $(T/273)^2 (p_s - p_a)$, in which the temperature term is not very important. The annual cycle of evaporation is, therefore, primarily dependent upon changes in $p_s - p_a$. The value of p_a is the vapour pressure above the still layer and is given by (i) R.H. of air and saturation vapour pressure at the air temperature, or (ii) saturation vapour pressure at the dew point. The value of p_s is the water vapour pressure at the soil

surface, and when the surface is at 100 % R.H., p_s is the saturated vapour pressure of air at the *temperature of the soil surface*. To obtain an estimate of p_s we make two assumptions about soil surface conditions. (a) The moisture content is assumed to be sufficient to keep the air in the surface at 100 % R.H.; (b) the mean surface temperature is given by the mean air temperature (T). We shall discuss later the effects of the errors introduced by these approximations. They lead to an expression for the evaporating power in terms of air conditions:

$$\text{Air evaporating power} = (p_T - p_a) (T/273)^2 = p_T (1.00 - \text{R.H.}) (T/273)^2,$$

where p_T is the saturated vapour pressure of air at T° K. Although $p_T \neq p_s$, it seemed worth enquiring how evaporation varies with

$$(p_T - p_a) (T/273)^2.$$

In Fig. 9 have been plotted the daily rates of evaporation against the evaporating power. The ordinates have been obtained from the constants of Table 8 and represent $R - D_{20}$ divided by the number of days in the month: these are means for the period 1878-1932. p_T and T have been obtained from the corresponding data (Koshal's Table 1), but the R.H. is the monthly mean for the 11-year period 1927-37. The variance in R.H. for a given month is not very great and the 11-year means are probably a good index to the corresponding means for the longer period. Koshal's equations have been used to correct the soil ordinates to the standard rainfall of 2.431 in. per month. The correction chiefly affects the summer months, for three of which a Sahni correction may also be made by extrapolating his data for shorter periods. To indicate the order of magnitude, the measured value, the Koshal corrected value and the Sahni corrected value are given (in. per day) for June and July, months with mean rainfall respectively less and greater than the annual monthly mean:

	Measured $R - D$	Koshal corrected	Sahni corrected
June	0.0542	0.0583	0.0602
July	0.0634	0.0594	0.0586

The Sahni correction is the greater, and as it may not be safe to extrapolate his data to 30-day periods, the Koshal-corrected points have been used in drawing the curve. This is smooth and shows no trace of a loop, i.e. the rate of evaporation is a unique function of evaporating power which is thus a more satisfactory parameter than mean air temperature. On the figure have been plotted data for the rate of evaporation from an open water surface. They have been derived from

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tables given by Latham (1909) and are 30-year means (1879-1908). The evaporating power is calculated from the temperature of the water in the evaporation tank and that of the dew point. There are several approximations involved, similar in kind to those made in computing the soil data. The readings are 9 a.m. values and not true daily means;

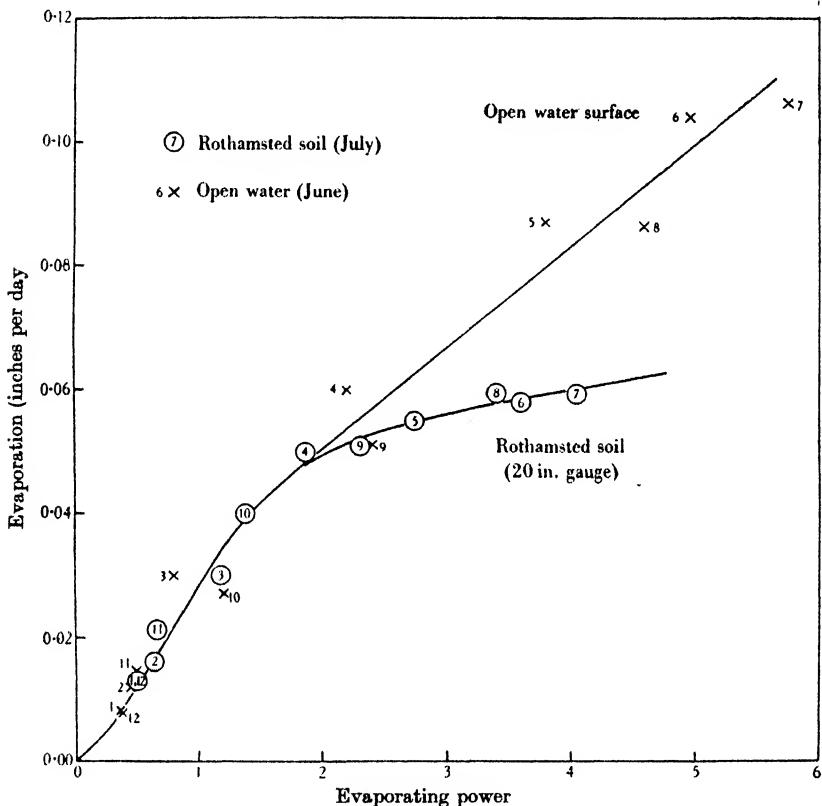


Fig. 9. Seasonal variation of mean daily evaporation from open water and fallow soil.

the effects of sunshine are ignored and will be most marked in summer when the sun warms the bottom of the tank; the water temperatures are not surface temperatures, and anomalous effects might be anticipated in winter when the air temperature is below 4° C. Finally, there may be a rainfall correction necessary, as the changes in tank level were compensated for increments due to rain, presumably measured on a rain gauge of smaller area. The Rothamsted 1/1000 acre rain gauge usually

records up to 10 % more rain than the 5 and 8 in. gauges and it may be that Latham under-estimated the amounts added in rain.

The winter groups of the two sets of data are fitted by the same curve, which, except near the origin, is a straight line, indicating that during the six months October to March the evaporation from fallow soil is practically the same as that which would take place from an open water surface exposed to the same meteorological conditions. There is no extensive drying of the surface during this season and between falls of rain the water supply from below is sufficient to keep the surface at 100 % R.H.

If the abscissa were an exact measure of $(p_s - p_a) (T/273)^2$ one would expect the open water curve to be a straight line passing through the origin. The curvature near the origin is probably due to the surface temperatures being less than that of the air; the surfaces will tend to take up the wet bulb temperature, and thus the winter evaporating power will be overestimated. In the water tank, convection will normally tend to equalize temperatures, but this action will be ineffective during long periods in winter when the air temperature is below 4° C. In the soil, the variations in soil surface temperature are probably similar to those observed by Keen & Russell (1921) at 6 in. They found that the winter soil temperatures at these depths were generally lower than, and out of phase with, the air temperature. Although the soil surface and the air form parts of a coupled system whose temperatures must be inter-dependent, it is apparent that in winter months a small change in either temperature may produce a relatively large change in evaporating power, and because of phase differences in the diurnal variations very different amounts of evaporation will be possible in periods which have the same rainfall, mean air temperature, mean relative humidity, and mean wind velocity. Hence the large scatter in the winter points in Fig. 7.

During summer months the evaporating power is sufficiently large to cause drying out of the soil surface between showers. The evaporating power will be greater than has been calculated because the mean soil surface temperature is higher than the mean air temperature by an amount which is probably dependent on the amount of sunshine. The records of Keen & Russell show that this temperature difference is marked at 6 in. While the surface is wet after a shower, evaporation will be rapid and the layer of 100 % R.H. will quickly retreat into the soil, so that for the greater part of summer months the resistance to evaporation imposed by the soil may be as great or greater than that due to the still layer. Fluctuations in air temperature will then have very little

effect and the total amount of evaporation in a summer period will be primarily dependent upon the number of times the surface is wetted during the period; in general this means that the more rain there is, the greater will be the total evaporation, but, in months of equal rainfall, there may be wide variations depending upon rainfall distribution. Corrections for variations in meteorological conditions will, therefore, have little or no effect on the scatter of summer values of evaporation (Fig. 7). Thus in Sahni's analysis of the residuals the minor role played by atmospheric conditions is reflected in the insignificant correlation with mean air temperature and the indeterminate nature of the regressions on humidity and wind velocity.

The conditions necessary for a successful description of evaporation from soil are now clear. Although other variables are important, the primary objective must be the evaluation of the term $(p_s - p_a)$ in the general diffusion equation (p. 102); the winter data of Fig. 9 show that where a reasonable rough approximation can be made the dependence of the rate of evaporation on evaporating power is as expected. Even in summer, where the approximation ceases to be reasonable, the evaporating power is still a more useful parameter than mean air temperature if empirical correlations are being sought. The inadequacy of the summer approximation may be regarded as due either to the retreat of the layer at 100 % r.h. into the soil, so introducing a soil resistance to evaporation, or to the reduction of the r.h. of the soil surface air below 100 %; from both conceptions one finds that p_s is less than its maximum value—assumed maintained in calculating evaporating power—for long periods in summer. The direct measurement of p_s and p_a will be difficult; an indirect approach through measurement of temperature and humidity of the air in and above the soil surface may be possible. This will involve four variables instead of the two used by Crowther and Koshal and until all four can be included in a statistical survey incorrect conclusions may be drawn from analyses based on partial knowledge of the factors involved.

SUMMARY

1. Study of the automatic records shows:

- (a) There is a seasonal change in the drainage response after rain which can be almost wholly ascribed to viscosity changes arising from seasonal changes of soil temperature (p. 77).
- (b) Evaporation occurring after a fall of rain has no measurable effect on the drainage response to that rain (pp. 78, 87).

(c) The maximum drainage rates for the 20 in. gauge are much larger than those for the deeper gauges. The maxima change seasonally and are again primarily dependent on viscosity (p. 78).

(d) There is no marked change in the field capacity of the gauge during the year. The air-filled pore space at field capacity may change by about 15 % of its average value (p. 81).

(e) The air-filled pore space at field capacity averages about 1.6 % (0-20 in.) and 0.25 % (20-60 in.). This order of magnitude is confirmed by measurements of Lawes & Gilbert (p. 81).

(f) Drainage begins some time after rain starts, the delay being normally due to (i) the need to bring the soil up to field capacity, and (ii) the finite time required for water to move through the gauge (p. 83).

(g) Abnormal drainage may occur when (i) rain falls very heavily and air is entrapped, or (ii) the soil is partially frozen (pp. 84, 86).

(h) Natural periods can be chosen over which $R - D$ can be equated to the evaporation. Good approximations are: periods of a year, six months, or long series mean of individual calendar months. A single calendar month will rarely approximate to a natural period (p. 87).

2. The daily totals reveal:

(a) The 20 in. gauge rarely loses more than 0.7 in. of water by evaporation. This corresponds to an air content of 5 %. The corresponding figure for cultivated soil is c. 8 % (p. 90).

(b) Most of the summer evaporation occurs soon after the end of the rainfall (p. 91).

(c) Total summer drainage is very much dependent on rainfall distribution; winter drainage is little affected (p. 92).

3. There is a leak in the casing of the 40 in. gauge. There may be a slight fault in the walls of the 60 in. gauge (p. 93).

4. A brief survey of the Pusa daily totals indicates that drainage is facilitated by root-holes, etc., the rate of drainage appears to be slower than at Rothamsted, and the extent of the water loss between monsoons very much greater (p. 94).

5. The seasonal totals show:

(a) Annual evaporation is nearly constant; it increases with increasing rainfall (p. 96).

(b) Summer evaporation is about two to three times as great as that in winter; it is dependent upon rainfall whereas winter evaporation is independent of rainfall.

(c) There is no evidence of a secular change in the seasonal evapora-

tion; a secular change in the annual evaporation is due to a change in rainfall distribution (p. 98).

(d) Winter evaporation is not dependent upon the nature of the preceding summer weather (p. 98).

(e) The equations of Koshal and Crowther are reconsidered as regressions of $R - D$ (deficit) on R and T . They are shown to be complementary and indicate a seasonal change of deficit. The use of natural periods does not improve the significance of the Koshal regression on mean air temperature. His explanation of the non-significance is rejected (p. 100).

6. Evaporation is discussed on a physical basis in terms of the water vapour pressure gradient in the air immediately above the soil surface:

(a) In winter the soil does not dry at the surface. Winter evaporation is, therefore, much the same as would be obtained from a water surface and extra rainfall does not affect it.

(b) In summer the surface remains moist only for a short time after rain has fallen; the air gradient is then much steeper than in winter. For the rest of the time the surface is drier and there is also a vapour pressure gradient in the soil. Hence (i) there is more rapid evaporation while the surface is wet, (ii) the total amount of evaporation is dependent upon both total rainfall and on its distribution in time, (iii) the later stages of evaporation are more dependent upon soil conditions than on air conditions, and (iv) the total evaporation is much less than from open water (p. 105).

(c) An adequate description of evaporation in all seasons may be obtained from knowledge of $(p_s - p_a)$, the difference in the partial pressures of the water vapour of the air in and above the soil surface. This term is the most important in the general evaporation equation

$$dq/dt = D_0(T/273)^2 (p_s - p_a)/l,$$

and the apparent anomalies in previous statistical treatments are attributed to the impossibility of representing changes in $(p_s - p_a)$ in terms of changes of rainfall and mean air temperature alone.

Our survey, although covering broadly the period 1870-1940, has been chiefly concerned with the records of more recent years, during which the observations have been in the care of Mr W. C. Game. To him and to his various assistants during the past 30 years we offer our thanks for the consistently high standard of observation they have maintained.

practice. These are by no means watertight compartments; different soils, for example, need different varieties, and the introduction of a new variety will affect the agricultural practice, and the variety prove more or less resistant to disease and damage than the one used formerly. Soil and climate do not lend themselves to investigation, being fixed and immutable for any given place. A consideration of these two factors will determine the best wheat growing districts, but is irrelevant in a study of the possible methods of increasing yield per acre. Diseases and pests is a separate subject, immensely important in determining yield, but calling for specialized study; and plant breeding a subject for the botanist rather than the agriculturist. Agricultural practice, then, taken in the widest sense, seems to be the starting point. This in itself covers a very wide range, and demands an investigation of rates of sowing, evenness of sowing, manuring, seed-bed preparation and so on; it also, inevitably, overlaps into the other categories of what is, at the best, a rather arbitrary division.

The farmer may carry out operations at various stages of the growth which may affect the crop. Seed-bed treatment, for example, may be expected to modify the number of plants, and treatment in the spring may govern tillering. The effect of any such treatment on the final yield is ultimately required. In this two problems are involved: first, what is the effect of the treatment on the wheat plant at the time of application, and as a direct result of treatment? second, how and to what extent is the development of the plant at that stage reflected in the final yield? A combination of these two factors will give the answer to the practical man's question, "How does the treatment affect the final yield?"

These considerations demand an analysis of yield. Whatever form this analysis takes, there must be some starting point on a "per unit area" basis. The number of seeds sown per acre is the true basic factor, but this is very laborious to measure. In practice, the number of plants per acre, or plant density, is a more suitable attribute. It must be realized that differentiating effects, such as the fineness of tilth and the damage done by birds, will have previously affected the crop. If, then, plant density is taken as the basic measure on a per unit area basis, it is necessary to investigate, first, to what extent variations of plant density occur in representative fields, and secondly, how any attribute considered varies in different plant densities.

The fluctuations of plant density from point to point in the wheat field are very considerable. It is true that large bare patches are not often seen, but detailed counts readily show a large variation of plant

density in successive foot lengths of the same drill row. The simplest way of measuring the intensity of relationship between the number of plants and the most important attribute, yield, in field plots is by using the correlation coefficient. The regression coefficient and the equation of the regression line may also be obtained, and an estimate of the yield for any specific plant density may be calculated by simple substitution. Such correlations and regressions are normally positive and significant. From this it has been argued by Doughty & Engledow (1928) and Anon. (1926) that considerable potential yield is lost in those areas where a low plant density occurs, and that spacing is a limiting factor to yield.

From this it seemed as if an increase of yield per acre might be brought about by two methods:

- (1) Increasing the evenness of seeding. This is mainly a problem for the manufacturers of seed drills.
- (2) Increasing the seed rate, since areas of high plant density appear to give high yields.

The relation between plant density and yield is entirely a matter of plant competition. Such competition may be divided into two sections. There is, first, the "internal" competition between plants in small individual units, such as foot lengths of drill row, which has been examined in great detail by Engledow and his co-workers (1925-30). Secondly, there is the "external" competition due to plants in the surrounding units, which has only been examined under Australian conditions (Fairfield-Smith, 1937). It will be shown that under English conditions such competition is operative and affects the yield adversely.

The yield per plant is determined by the competition due both to plants within the unit and plants around the unit; the seed rate, since it implies, ideally, even plant density, determines the size of both forms of competition to some extent. The role of competition in determining the optimum seed rate will be discussed separately in a later paper.

Evenness of seeding is the secondary problem of fluctuation around a mean plant density determined by the seed rate. Such fluctuation causes loss of yield because the loss in areas of low plant density is greater than the gain in areas of correspondingly high plant density, and it will be shown that it affects the total yield very little.

The studies in this paper owe their inspiration to the work of Engledow and his co-workers, and are the logical continuation of it. Their aim is to establish the part played by propinquity, and to measure the intensity of the effect. In § 2(a) the part played by external competition is shown, and measured by a method involving several approximations. In § 2(b) the

effect of competition at various plant densities is shown, and this leads to a discussion of its nature and causes in § 3. The practical problem of evenness of seeding is discussed in § 4, and it is shown that the loss of yield due to uneven seeding is negligible in the three instances investigated.

2. THE MEASUREMENT OF PROPINQUITY

(a) General investigation

Although it has been recognized that the decrease in yield in any particular small area containing few plants is recompensed by an increase of yield in the adjacent areas, direct inquiries into the size of the compensation are few. Fairfield-Smith (1937) has shown that competition exists, and postulated that the often-recorded correlation between plant number and yield might well be spurious and due to the relationship between yield and a factor q , the ratio of number of centre plants to number of surrounding plants. The problem has also been investigated for maize (Kiesselbach, 1923), potatoes (Stewart, 1919) and sugar beet (Garner & Sanders, 1939), and the results obtained all demonstrate that compensation occurs when a "gap" reduces the intensity of competition. The increase of yield due to reduced competition has also been investigated indirectly in the many studies of border effects.

The work outlined in this section was planned to show that a compensatory effect exists in fields of wheat. The data obtained from two uniformity trials, each consisting of 7200 "units" of 6 in. of drill row, were used in the investigation. The agricultural details of the trial have been recorded in a previous paper (Hudson, 1939). With root crops the position is simple; each site in the surrounding ring is either occupied or unoccupied; with wheat, however, a considerable range of surrounding plants may occur, and the presence of competition is best shown by a negative correlation coefficient between the yield and the number of surrounding plants. The size of the correlation then measures the intensity of the competition.

Propinquity may be considered in two ways—either as the effect of a central unit on surrounding units or the effect of surrounding units on a central one. In this investigation the effect of variable plant density is considered in the latter way. Throughout it is assumed that the correlations and regressions of the sum of "surrounding plants"

$$A_{s_1} + A_{s_2} + A_{s_3} + A_{s_4} = A_s \text{ (Fig. 1)}$$

on the yield (G_c) of the central unit form an adequate basis for the measurement of the competition effect. Thus a negative correlation

between the yield of the central unit, G_c , and the sum of the surrounding plants, A_s , shows the existence of competition and measures its intensity.

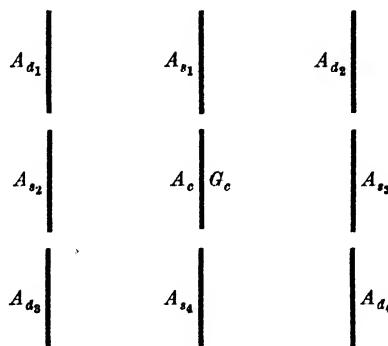


Fig. 1.

To obtain a general measure of these correlations the computations made with the aid of Hollerith equipment were available (Comrie *et al.* 1937). The method used to obtain the sums of squares of all characters for all sizes of areas was so planned that it was easy to get the sums of products between each and every character for all sizes of plots. From these the correlation and regression in each instance were obtained. In particular, the correlations between plant number and grain weight were obtained from the population of (a) units (6 in. lengths), (b) 12 in. lengths, i.e. pairs of units in a "linear" direction, (c) 6 in. pairs—pairs of units in a "lateral" direction (Table 1). The correlation in pairs of units is the aggregate of correlations between plant number and grain weight in the same unit, between adjacent plant numbers, between adjacent grain weights, and between plant number in the one unit and grain weight in the adjacent unit of the pair. This last "cross-correlation" measures a propinquity effect. Using this principle we can determine an expression giving a "competition correlation" in terms of the correlation between

Table 1. *Correlation between plant number and yield, calculated from 6 in. singles, 6 in. pairs and 12 in. singles*

Basis of calculation	1934-5*	1935-6
6 in. singles	+0.493	+0.453
6 in. pairs	+0.396	+0.397
12 in. singles	+0.410	+0.364

* In 1934-5 the correlations were calculated from one strip, chosen at random. In this instance the sums of products could not be formed by the Hollerith equipment. In other cases where full calculations were made the loss in point of error due to using a limited amount of the data could be tested. It was found to be negligible.

plant number and yield for both units (r_{AG} units) and for pairs of units (r_{AG} pairs), the correlation between adjacent plant numbers in units (r_{ab}) and the correlation between adjacent grain weights in units (r_{xy}):

$$r_{\text{competition}} = \sqrt{2} \{ r_{AG} \text{ pairs} \sqrt{\{(1 + r_{ab})(1 + r_{xy})\}} - r_{AG} \text{ units} \}. \quad (1)$$

r_{AG} pairs and r_{AG} units are already known, and $(1 + r_{ab})$ and $(1 + r_{xy})$ may very readily be calculated as follows. If α be the sums of squares for units, and β the sums of squares for pairs of units expressed on a basis of units, then

$$(1 + r_{ab}) = \frac{2\beta}{\alpha}. \quad (2)$$

This expression gives an easy way of calculating the effect of competition either laterally ($r_{(a_{s_2}+a_{s_3})ac}$), using units and 6 in. pairs, or "linearly" ($r_{(a_{s_1}+a_{s_4})ac}$), using units and 12 in. singles. The two correlations may be combined to give an estimate of the total competition correlation as defined on p. 119 by using the relationship

$$\frac{r_{\text{lateral competition}} + r_{\text{linear competition}}}{\sqrt{2}} = r_{\text{total competition}} = r_{A_s G_c}$$

These equations cannot be taken as absolute identities, since four assumptions have been made in deriving them. These are:

- (1) That the sum of squares of the 3600 even units is equal to that of the 3600 odd units.
- (2) That the correlations $r_{a_{s_1} a_{s_4}}$ and $r_{a_{s_2} a_{s_3}}$ are 0.
- (3) That the correlation $r_{(a_{s_1}+a_{s_4})(a_{s_2}+a_{s_3})}$ is 0.

It is felt that little is lost by these assumptions, and that the estimate of the size of the competition correlation calculated by this method will give a very good indication of the nature and size of the effect.

Computed by this method the "linear" competition correlation in 1935-6 between surrounding plants and yield is -0.1335 . The true effect is, however, masked by correlations of plant number in adjacent units (linear, $+0.0347$; lateral, $+0.0916$) and grain weight in adjacent units (linear, -0.0799 ; lateral, $+0.0195$), and the partial correlations with plant number of the central unit (A_c) eliminated give a truer picture. This partial correlation for linear competition is -0.2114 , that for lateral competition -0.0966 , and that for the total competition -0.2178 . To find the significance values of $t = \frac{r}{\sqrt{(1-r^2)}} \sqrt{(n^2-2)}$ are calculated (Fisher, 1938). Assuming that $n^2 = 7200$, the values of t are 17.18, 7.73 and 20.33 for linear, lateral and total competition respectively. All these values

are easily significant. The data of 1934-5 give similar but less decisive figures. The linear correlation, corrected for plant number, is -0.0807 , the lateral -0.1106 , and the total -0.1353 . All these correlations are significant, the values of t being 6.96 , 9.44 and 11.58 respectively. This shows that there is a significant competition effect—that the greater the number of plants in the surrounding units, the less the yield. The effect of the lateral units is considerable and significantly greater than that of the linear units in 1935-6, the difference of the transformed correlation being -0.121 ± 0.0236 —a fact that will be discussed later. In 1934-5 the difference, 0.0299 ± 0.0168 , is insignificant. It is interesting to compare these correlations with those between plant number of the central unit (A_c) and yield at the centre (G_c), which affected the yield to the extent of $+0.4927$ in 1935-6 and $+0.5566$ in 1934-5.

(b) Detailed investigation

A more detailed investigation of the nature and intensity of the competition effect was carried out on three sets of data obtained by sampling from the original data. The object of these experiments was to find the competition correlations and regressions for varying classes of central plant numbers, and, vice versa, the correlations and regressions of plant number on yield for various intensities of competition. In every case the plant number and grain weight for a series of central units of 6 in. of drill row (A_c , G_c) was observed, together with the number of plants in the adjacent units linearly (a_{s_1} and a_{s_4}) and laterally (a_{s_2} and a_{s_3}). Three sets of data were obtained by sampling, and these will be referred to as Exps. A, B and C. The data for Exp. A were obtained from the main experiment of 1934-5 and were drawn from 1381 areas; those for Exp. B were drawn from the high-yielding area of the experiment for 1935-6 (937 areas), and those for Exp. C from the low-yielding area of the experiment for 1935-6 (1515 areas). The aim of the sampling was to obtain an equal number of areas for each class of central plant number—100 in the case of Exps. A and C, and 60 in the case of Exp. B. It was easy to obtain this amount in those classes where the plant number was near the mean for entire population, but in the more extreme classes it was impossible to obtain the full amount even by taking every area available. Table 2 shows, for the three experiments, the number of areas in each plant number class, together with the total number of areas available for sampling, the sampling percentage, and the means of yield and plant number (as measured by the average of all surrounding plants) per unit.

Table 2. Description of Exps. A, B and C

Plant no. class	Experiment		
	A	B	C
2	38	32	59
3	66	31	100
4	77	40	100
5	100	53	100
6	100	60	100
7	100	60	100
8	100	60	100
9	100	60	100
10	100	60	100
11	100	60	100
12	100	60	101
13	100	60	100
14	100	60	100
15	100	57	100
16		58	100
17	100	48	77
18	—	32	78
19	—	46	—
20	—	—	—
Total no. of areas taken	1381	937	1515
Total no. of areas available	5690	2088	3440
Sampling percentage	24.27	44.88	44.04
Mean yield per unit in g.	7.096	9.8664	5.2419
Mean plant no. per unit	8.781	11.017	10.752

In collecting the samples no attempt was made to prevent areas overlapping, and it was quite possible for, say, the a_{s_1} of one area to be the a_c of another area. Neither was the method of sampling strictly random. For each class the total number available was known and it was easy to calculate whether every other area, every third area or every fourth area, etc. encountered in looking over the original sheets of data would provide the correct number of samples. The samples were then drawn in this regular manner, and any additions necessary to make up the final total to 60 or 100 were chosen at random. The corresponding values of the grain weight and the total number of surrounding plants ($a_{s_1} + a_{s_2} + a_{s_3} + a_{s_4} = a_s$) were noted for each area. The linear regression, $b_{ac}a_s$, and curved regression, c , were calculated for each plant number class by Isserlis's (1927) method, which allows for the weighting of each individual mean, and their significances tested. In addition the correlation coefficients, $r_{ac}a_s$, were calculated. The values of "b", "c" and "r", together with the mean yield and mean number of competing plants for each class of plant number in Exps. A, B and C, are given in Tables 3-5.

The size of the negative correlation coefficient in these tables provides a measure of the intensity of the competitive effect. The many significant

coefficients confirm the fact that plant competition is one of the factors that control final yield—a conclusion already drawn in § 2(a), by the more approximate method. Most of the linear regression coefficients are also significant, but the curvature is negligible, being only significant in

Table 3. *Exp. A. "b", "c" and "r" for competition \times yield for varying plant numbers*

Plant no.	Size of sample	Mean yield	Mean competition	"b"	"c"	"r"
2	38	2.71	30.05	-0.0565	+0.00212	-0.281‡
3	66	2.88	33.39	-0.0515‡	+0.00024	-0.282‡
4	77	3.81	33.60	-0.0356	+0.00220	-0.141
5	100	5.24	34.97	-0.0910	+0.00041	-0.357
6	100	5.59	34.65	-0.0922	+0.00234	-0.358
7	100	6.25	36.78	-0.1478	+0.00096	-0.464
8	100	6.05	35.30	-0.0901	+0.00484	-0.330
9	100	6.56	34.77	-0.0993	+0.00301	-0.332
10	100	8.15	33.75	-0.1404	+0.00599‡	-0.368
11	100	7.63	37.19	-0.1408*	+0.00165	-0.411*
12	100	8.47	35.27	-0.1546	+0.00127	-0.447
13	100	8.29	36.23	-0.1451	+0.00147	-0.386
14	100	9.54	35.40	-0.1761	+0.00024	-0.352
15	100	10.06	37.45	-0.2220	+0.00960‡	-0.441
16	100	10.39	34.04	-0.1694	+0.00298	-0.329
17						
Mean		7.101	35.125	-0.1026*		-0.2371*

In this and subsequent tables * denotes significance at 0.1 % point.

† denotes significance at 1 % point.

‡ denotes significance at 5 % point.

Table 4. *Exp. B. "b", "c" and "r" for competition \times yield for varying plant number*

Plant no.	Size of sample	Mean yield	Mean competition	"b"	"c"	"r"
2	32	3.44	39.59	+0.0028	-0.0131‡	+0.013
3	31	4.18	40.94	-0.0432	-0.0010	-0.207
4	40	4.86	40.45	-0.1038‡	+0.0092‡	-0.319‡
5	53	6.94	42.17	-0.0287	+0.0021	-0.068
6	60	8.25	44.78	-0.0466	+0.0020	-0.119
7	60	8.51	42.05	-0.1105‡	-0.0011	-0.316‡
8	60	9.33	40.33	-0.1265†	-0.0025	-0.357*
9	60	9.47	43.00	-0.1381†	+0.0040	-0.348*
10	60	10.08	45.55	-0.0219	-0.0003	-0.067
11	60	10.97	42.90	-0.1310†	-0.0026	-0.379*
12	60	10.06	45.80	-0.1151‡	+0.0054	-0.257‡
13	60	11.46	45.60	-0.2360*	+0.0040	-0.483*
14	60	11.61	45.35	-0.1220‡	+0.0090‡	-0.293‡
15	57	12.16	45.04	-0.0409	+0.0029	-0.071
16	58	12.70	46.24	-0.0706	-0.0012	-0.215
17	48	12.66	46.06	-0.1107	+0.0040	-0.263
18	32	14.21	47.84	-0.0800	-0.0078	-0.198
19	46	12.29	46.83	-0.0156	-0.0094	-0.036
20						
Mean		9.8664	44.068	-0.0463†		-0.1025†

Table 5. *Exp. C. "r" and "b" for competition \times yield for varying plant number*

Plant no.	Size of sample	Mean yield	Mean competition	"b"	"r"
3	59	2.612	40.58	-0.0250	-0.210
4	100	3.312	38.49	-0.0703*	-0.363*
5	100	3.811	37.33	-0.0315†	-0.209†
6	100	4.130	41.38	-0.0392‡	-0.240†
7	100	4.527	41.73	-0.0688*	-0.383*
8	100	5.233	43.24	-0.0948*	-0.464*
9	100	4.810	44.15	-0.0826*	-0.442*
10	100	5.411	43.63	-0.0660*	-0.353*
11	100	5.354	44.99	-0.0685*	-0.356*
12	101	6.135	44.42	-0.0615†	-0.307*
13	100	6.121	44.37	-0.0819*	-0.365*
14	100	6.515	44.68	-0.0633†	-0.303*
15	100	5.958	44.81	-0.0361†	-0.208†
16	100	6.388	45.33	-0.0301	-0.137
17	77	6.715	42.82	-0.0451†	-0.249†
18	78	6.329	45.83	-0.0498‡	-0.272‡
Mean		5.2419	43.01	-0.0476*	-0.2250*

two cases in Exp. A and three in Exp. B, where it has both positive and negative values. Its variation has no relationship with the central plant number, and it has not even been worked out for Exp. C.

The variation of these coefficients throws light on the working of competition. In two of the three experiments the correlation is small and insignificant for very high and very low plant number classes; in the third there is a steady increase of the correlation within the range examined, with an indication that the maximum had been reached. Graphs showing the competition correlation for various classes of centre plant number were constructed direct from Tables 3-5, and the significance of the linear and curved regression tested. These significances are given in Table 6 in terms of Snedecor's *F* (1937) (variance of treatment \div variance of error), which leads to a direct test of significance. The curvature is significant in Exps. B and C, and nearly significant in Exp. A, proving that the effect of competition is greatest at medium plant densities in two cases, and suggesting it strongly in a third. The plant number at which competition is most intense (measured by differentiating the equation of the curve and equating with 0) is also given together with the correlation at that point.

The changes in intensity of competition may be explained by considering three types of plant density. First, in those units which have few plants, the actual number of plants present is the controlling factor. In the limit, if there are no plants, there will be no yield, whatever the competition, and if there are only two or three plants, then each indi-

Table 6. *Summary of results from Exps. A, B and C relating to the curves connecting the competition correlation and regression with the plant density class*

Result	Experiment					
	A		B		C	
	Correlation	Regression	Correlation	Regression	Correlation	Regression
Linear regression	+0.00950	+0.01056	+0.00149	+0.00205	-0.00454	-0.00024
<i>F</i> for linear regression	6.20†	62.40*	0.76	0.86	1.26	0.08
Curved regression	-0.00115	0.00000	-0.00297	-0.00132	-0.00269	-0.00068
<i>F</i> for curved regression	1.96	—	6.53‡	7.83‡	7.41‡	9.43†
Sig. <i>F</i> at 5% point is greater than		4.67		4.49		4.60
Sig. <i>F</i> at 1% point is greater than		9.07		8.53		8.86
Optimum plant no.	13.1			10.7		9.7
Competition correlation at optimum plant density	-0.443			-0.302		-0.368

vidual will be adequately supplied, and the loss due to competition will not be reflected in yield. The intensity of such competition is, therefore, immaterial, and the yield is controlled by the number of plants present in the central unit. As soon as the number of plants increases competition becomes operative, and so we find that, with a medium number of plants, the number of surrounding plants affects the yield. Finally, there is the stage when the plants in the unit become unduly crowded within the unit; in this case the plant competition within the unit becomes so intense that external competition becomes of secondary importance. This is shown by the insignificant negative correlation coefficients in high plant number classes. The reasons for the variations between the size of correlation and position of maxima in the three experiments will be discussed in § 5.

Further information was obtained from this series of data by re-classifying according to the amount of competition and investigating the relationship of plant number and yield in the various competition classes. The data were grouped for this purpose into sections of 4 surrounding plants, and figures analogous to those in Tables 3-5 are given in Tables 7-9. The correlations of plant number and grain weight are larger than those of competition \times grain weight, showing that the number of plants in a unit has a greater effect on the yield than the number of plants surrounding it. The presence of competition is, however, shown in each experiment by the steady decrease of the mean yields with increasing competition. A positive correlation between adjacent plant numbers is also shown by a steady rise of the mean plant number

Table 7. *Exp. A. "b", "c" and "r" for plant no. \times yield at different levels of competition*

Class of competition	Size of sample	Mean yield	Mean plant no.	"b"	"c"	"r"
-25	197	8.45	8.605	+0.752	-0.0121	+0.722
26-29	195	8.20	9.872	+0.602	-0.0163	+0.611
30-33	218	7.43	9.622	+0.542	-0.0296†	+0.591
34-37	212	7.19	9.495	+0.501	-0.0197	+0.552
38-41	229	6.34	9.746	+0.461	-0.0125	+0.574
42-45	164	6.28	9.732	+0.433	-0.0007	+0.530
46-	166	5.70	9.994	+0.423	-0.0202	+0.606
Mean	1381	7.101	9.581	+0.576*	+0.531*	

Table 8. *Exp. B. "b", "c" and "r" for plant no. \times yield at different levels of competition*

Class of competition	Size of sample	Mean yield	Mean plant no.	"b"	"c"	"r"
-33	154	10.76	9.64	+0.560	-0.065*	+0.547
34-37	102	10.63	10.07	+0.588	-0.058†	+0.531
38-41	135	9.89	10.24	+0.546	-0.031†	+0.534
42-45	145	9.11	10.40	+0.579	-0.021	+0.599
46-49	122	10.38	11.41	+0.664	-0.026	+0.599
50-53	119	8.99	11.32	+0.525	+0.004	+0.570
54-	160	9.43	11.90	+0.416	-0.011	+0.433
Mean	937	9.8664	10.721	+0.499*	+0.497*	

Table 9. *Exp. C. "b", "c" and "r" for plant no. \times yield at different levels of competition*

Class of competition	Size of sample	Mean yield	Mean plant no.	"b"	"c"	"r"
-25	74	6.258	8.616	+0.342	-0.0850*	+0.485
26-29	99	5.868	9.364	+0.293	-0.0237†	+0.508
30-33	140	5.682	9.843	+0.285	-0.0188†	+0.543
34-37	172	5.691	10.227	+0.267	-0.0358†	+0.426
38-41	201	5.260	10.060	+0.326	-0.0202†	+0.576
42-45	218	5.169	10.422	+0.234	-0.0235†	+0.471
46-49	190	4.878	10.916	+0.198	-0.0139	+0.417
50-53	162	4.926	11.475	+0.272	-0.0134	+0.547
54-57	117	4.860	11.650	+0.262	-0.0065	+0.525
58-	142	4.564	11.380	+0.271	-0.0036	+0.573
Mean	1515	5.2419	10.465	+0.248*	+0.464*	

with increasing surrounding plants in two of the experiments. The behaviour of the correlations and linear regressions (Table 10) is difficult to understand, since the trends are extremely irregular, but the negative regression of the curvature \times the class of competition, showing that the more intense the competition the smaller the curvature, is a finding that is of importance in considering the nature of competition.

Table 10. *Summary of results from Exps. A, B and C relating to the curves showing the correlation, regression and curvature of plant no. \times yield at various densities of competition. Values of F*

	A	B	C
Linear regression \times plant density: Linear	1.57	55.03†	3.26
Curvature	1.14	2.89	2.60
Curvature \times plant density: Linear	18.27‡	1.18	17.82‡
Curvature	0.49	4.26	4.05
Correlation \times plant density: Linear	0.73	4.73	2.73
Curvature	1.29	2.32	24.11†
Sig. F: 5 %	7.71	7.71	5.59
1 %	21.20	21.20	12.25

3. THE NATURE OF PROPINQUITY

The succession of various plant communities on different types of land has long attracted the attention of the ecologist. Such succession is primarily governed by competition between species and species. In agriculture competition may conveniently be considered under two headings. First, there is the competition between different species, found in the competition between the cultivated crop and the weed in the same field; secondly, there is the competition between plants of the same species, which is governed by the spacing.

Competition in cultivated crops takes place for (a) water, (b) soil nutrients, (c) soil air, and (d) light. The first three factors involve root competition, the last aerial competition. The relative degree of importance of each of these four factors has been investigated by various workers. The majority of earlier work has been done on the continent, and is extensively summarized by Clements, Weaver & Hanson (1929). The earliest work seems to be that of Sachs (1860) on buck-wheat, who showed that competition existed and surmised that it was for soil nutrient. Since then the problem has been investigated by Wollny (1881), Hellriegel (1883) and Mayer (1879), who paid particular attention to competition for light. Harrington & Pavlychenko (1935), working with Hare's Ear Mustard, which makes very little shade and so eliminates competition for light automatically, have more recently shown that root competition is of great importance.

The most comprehensive study of the problem has been made by Clements *et al.* (1929), working chiefly on the sunflower and on wheat under the dry conditions of the Middle West in U.S.A. They investigated the competition for water, nutrients and light, each factor being tested in turn whilst the other two were artificially held constant, and showed

that competition for water is by far the most important consideration, competition for nutrients of secondary importance, and competition for light of negligible importance. The authors, however, realize that the importance of various factors "depends primarily upon the relative quantity of each", and that "any one of the four factors, water, light, nutrients or soil air would become controlling when the relative supply of it was lowest".

The degree to which competition will affect the yield thus depends on the abundance of these four factors. Conditions in this country differ from those in America. Moisture conservation is less important, and it may well be that the competition for moisture is not of such supreme importance as it is in America. In the present investigation no attempt has been made to differentiate between the factors involved in competition between roots. There is, however, considerable evidence to show that root competition is more important than aerial competition, and this will now be reviewed.

In the study outlined in § 2(a) the total competition was finally obtained by combining the competition due to the lateral units (a_{s_2} and a_{s_3}) and the linear units (a_{s_1} and a_{s_4}), which had been separately computed. If the competition is beneath the ground, then the linear competition ought to be greater than the lateral competition. Those plants at the ends of the 6-in. lengths of drill row are *immediately* adjacent to the linear units a_{s_1} and a_{s_4} , and are only 6 in. away from the most distant plants in the adjacent units, yet they are $7\frac{1}{2}$ in. from the nearest plant laterally. The plants which are best situated, from the point of view of root competition, are 3 in. from the nearest linear unit and $7\frac{1}{2}$ in. from the nearest lateral unit. Thus the number of roots from the linear units will be more plentiful than the number from the lateral units, and the concentration of plants in the linear units will have a bigger effect than that in the lateral units. This assumes a small radius within which the roots of a plant can compete strongly. If the roots were able to compete at a distance, then the position of the lateral units would make their competing power more formidable. If the lateral units were to influence yield more than the linear it would be difficult to decide, without further experimentation, whether to attribute their effect to a big root range causing root competition, or to the shading effect; this problem, however, does not arise as the effect of the linear units is significantly greater than that of the lateral units in one year, and similar in the other (Table 11). The competition in terms of larger units is also given in this table, and shows, as might be expected, that the competition

of the lateral units is of greater importance and that of linear units of less importance when a larger unit is used.

Table 11. *Linear and lateral competition correlations (competition \times yield with plant number eliminated), total competition correlations, significance of differences between linear and lateral correlations, and partial correlations between plant number and yield with competition eliminated. Variation between blocks of 6 ft. \times 10 drill rows has been eliminated*

Unit	Lateral competition correlation	Linear competition correlation	Sig. of difference	Total competition correlation	Partial correlation of plants \times grain—competition eliminated
1934-5					
6 in. singles	-0.1106	-0.0807	Insig.	-0.1353	+0.5566
12 in. "	-0.1869	-0.0378	Lat. > Lin. at 1 %	-0.1589	+0.5027
18 in. "	-0.1311	+0.0344	Lat. > Lin. at 1 %	-0.0683	+0.4649
24 in. "	-0.1837	+0.1397	Lat. > Lin. at 1 %	-0.0622	+0.4475
36 in. "	-0.2338	+0.0692	Lat. > Lin. at 1 %	-0.1164	+0.5052
1935-6					
6 in. singles	-0.0906	-0.2114	Lin. > Lat. at 1 %	-0.2178	+0.4972
12 in. "	-0.1356	-0.1062	Insig.	-0.1710	+0.3728
18 in. "	-0.1701	-0.0922	Lat. > Lin. at 5 %	-0.1854	+0.3387
24 in. "	-0.1638	-0.0274	Lat. > Lin. at 1 %	-0.1352	+0.2879
36 in. "	-0.1850	-0.0301	Lat. > Lin. at 1 %	-0.1528	+0.2572

4. THE EFFECTS OF PROPINQUITY. EVENNESS OF PLANT

The average number of plants in any field is determined by the seed rate and percentage survival. Although the percentage survival varies considerably from field to field, it may be assumed to be constant for any one field. Thus the variation in number of plants from place to place in the same field is largely decided by the number of seeds sown.

Any area of low plant density must be balanced by a corresponding area of high plant density. If the corresponding area does not occur, then the average number of plants is reduced, and the problem becomes one of seed rate and not evenness of seeding. Such fluctuation of plant density from place to place has been observed many times, and investigated in detail by Engledow (1926). It is chiefly caused by the drill, although damage done by birds and roughness of the seed bed may also have effect. It has led to studies of drill mechanism by Engledow (1926), Rayns (1930), and Davies (1931). In these trials the evenness of distribution of wheat by various types of drills was examined at different rates of sowing, but no attempt was made to correlate the degree of evenness with the final yield. Such trials have, however, been made by Forster & Vasey ((unpublished) quoted by Fairfield-Smith, 1937) and

Fairfield-Smith (1937) in Australia. The first of these showed that evenly hand-sown plots had 13 % greater yield than drill-sown plots, but the significance of this increase is not available. Fairfield-Smith (1937) investigated even and uneven spacing with foot lengths of wheat, and found that an uneven plant gave a significantly greater yield per plot than an even plant.

The total loss of yield resulting from uneven seed distribution is best illustrated from first principles. In Fig. 2 a typical graph of plant

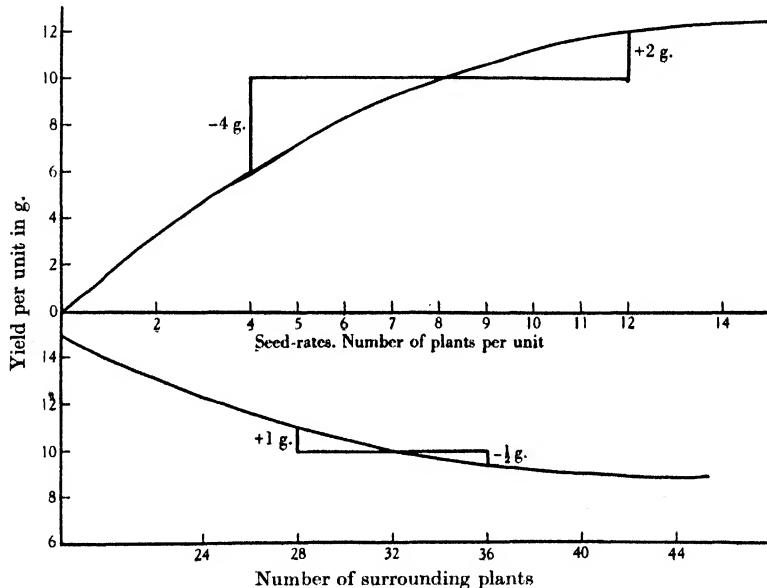


Fig. 2. To illustrate the theory of loss of yield due to unevenness of plant.

number \times yield for a population whose mean plant number is eight plants per foot is shown. If there had been no variation of plant density then each foot length would have had eight plants in it, and the average yield would be 10.0 g. Now consider the yield if a *single* foot length had four plants. To retain the plant density unaltered this means that a unit with twelve plants in it must also occur. The four plant foot length would have a considerably lower yield, say 6 g., but the twelve plant foot length would only have a yield of say 12 g. Thus, considering plant number only, there would be a loss of 2 g. due to the variation of plant density. If we now consider the competition effect, which has a small positive curvature (Fig. 2), we shall have a mean competition of thirty-two surrounding plants,

1 ft. length with twenty-eight surrounding plants, and 1 ft. length with thirty-six surrounding plants. The decreased competition will cause an increased yield of say 1 g., and the increased competition a decreased yield of say $\frac{1}{2}$ g., giving a net *increase* of yield due to competition of $\frac{1}{2}$ g. Thus the net loss of yield due to unevenness of drilling will be $2 - \frac{1}{2} = 1\frac{1}{2}$ g.

This principle may be used to calculate the loss of yield due to irregularity of plant in Exps. A, B and C. For a series of plant densities at a given competition there is a series of corresponding yields. Similarly the same series of plant densities at a second intensity of competition (surrounding plants) will give a new series of yields. Such series can be built up into a three-dimensional surface, from which the yield (y) can be read for any combination of plant density (x_1) and density of surrounding plants (x_2). The equation of such a curved surface may be calculated, and is of the form

$$y = \alpha + b_1(x_1 - \bar{x}_1) + b_2(x_2 - \bar{x}_2) + c_{11}(x_1 - \bar{x}_1)^2 + c_{12}(x_1 - \bar{x}_1)(x_2 - \bar{x}_2) + c_{22}(x_2 - \bar{x}_2)^2,$$

where α is the optimum mean yield of the entire population, b_1 and b_2 the average linear regression of yield on plant number and surrounding plant number, c_{11} and c_{22} the average curved regression of yield on plant number and surrounding plant number, and x_1 and x_2 values of plant number and total of surrounding plants. The values of α , b_1 , b_2 , c_{11} , c_{22} , and c_{12} , a hybrid regression term, for Exps. A, B and C are given in Table 12, where \bar{y} represents the actual mean yield.

Table 12. *Values of various constants concerned with fitting the equation of the surface*

	A	B	C
\bar{y}	7.0967	9.8667	5.2419
α	7.264	10.234	5.480
b_1	+ 0.5324	+ 0.5523	+ 0.2725
b_2	- 0.1235	- 0.0937	- 0.0636
c_{11}	- 0.0144	- 0.0184	- 0.0198
c_{12}	- 0.0106	- 0.0363	- 0.0018
c_{22}	+ 0.0051	- 0.0033	+ 0.0014

The significance of the linear and curved terms was tested, and both were found to be significant at the 1 % point. The loss of yield due to irregularity of drilling is given by the expression

$$y - \alpha = - \{c_{11}(x_1 - \bar{x}_1)^2 + c_{12}(x_1 - \bar{x}_1)(x_2 - \bar{x}_2) + c_{22}(x_2 - \bar{x}_2)^2\}.$$

The standard error of this quantity may be calculated and the significance of the decrease tested.

The loss of yield per plot is related to the amount of variation (as measured by σ^2 , the variance of the plant number taken in 6 in. length units) by the equation

$$\text{Loss of yield per unit} = \{c_{11} + \frac{1}{4}c_{22}\} \sigma^2.$$

In this equation $c_{11} + \frac{1}{4}c_{22}$ is the regression of the graph giving the loss of yield for various variations in plant density. Its significance may be tested, and should be similar to the significance of the estimate of the total loss of yield.

The values for these various characters for Exps. A, B and C are tabulated in Table 13.

Table 13. *Exps. A, B and C. Data regarding loss of yield due to uneven "plant"*

	A	B	C
Loss of yield (g.)	230.0 insig.	343.8	330.8‡
S.E. of loss of yield	135.9	—	99.8
$c_{11} + \frac{1}{4}c_{22}$	0.00927	0.01516	0.01404
σ^2	18.12	24.20	15.55
σ/m	47.04	44.65	37.43
Actual yield (bushels/acre)	34.58	48.08	25.55
Optimum yield (bushels/acre)	35.40	49.87	26.61
Loss of yield (bushels/acre)	0.82	1.79	1.06‡
% loss of yield (bushels/acre)	2.37	3.72	4.17‡

These figures show that there is not always a significant loss of yield due to irregular drilling, and that the size of the loss is comparatively small in all the three experiments.

5. DISCUSSION

That the phenomenon of competition plays an important part in determining yield has long been recognized. More recently it has been shown that competition for light is negligible and root competition all important (Clements *et al.* 1929). In any small area of land there is a limited amount of soil nutriment, soil air, and soil water. If there is only one plant in the area the problem is simple, but normally the necessities of life have to be shared out between several plants, in addition to various weeds. The development of each plant, and eventually its yield, will depend on it obtaining an adequate share.

Competition will only become operative when the spheres of absorption of the roots overlap. In root crops, with wide spacing between plants, competition only begins when the plant has developed to some extent. With cereals, the plants are much closer together, and root competition will begin at a much earlier date in the history of the plant.

The average yield of each area—say 6 in. of drill row—is obviously determined to some extent by the number of plants there, since each plant within the unit is competing for nutriment in that area. The problem of external competition, or propinquity, is that of the effect on the aggregate of competition from adjacent areas. Since intensity of competition must be proportionate to some function of the distance between the competitors, the effect of propinquity is not so marked as the effect of internal competition on the unit plant. Competition is only operative when demand exceeds supply. The plants at that place where the units meet in the same drill row will be the first to compete “externally”, and competition between laterally adjacent units will begin when the roots have grown about $3\frac{3}{4}$ in. if the drill rows are spaced $7\frac{1}{2}$ in. apart. This fact accounts for the bigger competitive effect of linear units shown on p. 130. If there are few plants in the centre unit there should be adequate nutriment for each plant even if some is taken. Thus the nutriment, etc., that roots from adjacent units “steal” will not affect the yield of the central unit. Similarly, if the plants are very crowded, internal competition for nutriments will be very intense, the invading roots will not be able to obtain much nutriment, etc., and again the yield will be unaffected by the propinquity. At medium plant densities, however, competition is operative because the loss of nutriment, etc., is critical, and the plants are not there in sufficient number to prevent such loss. If competition is operative—that is, if the loss of nutriment, etc., is a determining factor of the yield of the centre unit—then the more plants there are in the surrounding units the greater will be the loss of nutriment and the lower will be the yield of the centre unit. Thus the effective intensity of competition is measured by the size of the negative correlation between the number of surrounding plants and the yield of the central area.

In Exps. A, B and C, which are summarized in Table 14, the intensity of competition has been computed directly, and is expressed as the “competition correlation” (p. 119). These correlations may be compared directly with the field conditions and the indirect estimates of competition intensity. The yield of these experiments, which is taken to be diagnostic of the amount of nutriments available, varies considerably. The two halves of the 1935–6 experiment (B and C) were on rich and poor soil respectively, whilst the fertility of the field on which Exp. A was situated in 1934–5 was intermediate. The land was particularly dry in the late winter and spring of 1935, but in 1936 there was considerable rainfall at this period.

The average competition is greatest in Exp. A, and least in Exp. B.

This shows that the lack of water in 1934-5 was more important than the lack of nutriments in 1935-6. The plant density at which the maximum competition correlation occurs was lower when nutrient was scarce (Exp. C) than it was when it was more plentiful (Exp. B). Since competition was most severe in Exp. A, it might be expected that intensity of internal competition would cause the maximum correlation to occur at an even lower plant density than in Exp. C (Hudson, 1941). This does not occur.

Table 14. *A comparison of Exps. A, B and C*

Comparison	Experiment		
	A	B	C
Mean yield in 9 ft. per 6 in. length	7.0967	9.8867	5.2419
Yield in bushels/acre	34.59	48.09	25.54
Plant density (plants per 6 in. length)•	9.58	10.72	10.47
Yield \times competition:			
Competition correlation coefficient	-0.237*	-0.103†	-0.225*
Total regression	-0.103*	-0.046†	-0.048*
Average correlation	-0.360	-0.240	-0.308
Maximum correlation	-0.443	-0.302	-0.368
Yield \times competition:			
Regression of regression \times plant no. class	+0.01056*	+0.00205	-0.00024
Curvature of regression \times plant no. class	0.00000	-0.00132‡	-0.00068†
Regression of correlation \times plant no. class	+0.00950‡	+0.00149	-0.00454
Curvature of correlation \times plant no. class	-0.00115	-0.00297‡	-0.00269†
Plant number at maximum correlation	13.1	10.7	9.7
Yield \times plant no.:			
Total correlation coefficient	+0.531*	+0.497*	+0.464
Total regression	+0.576*	+0.499*	+0.248
Average correlation coefficient	+0.598	+0.545	+0.507
Yield \times plant no.:			
Regression of curvature \times plant no. class	-0.00038	-0.00802‡	-0.00545†
Curvature of curvature \times plant no. class	-0.00006	-0.00032	-0.00077
Loss of yield (g.)	230.0	343.8	330.8
s.e. of loss of yield	135.9	—	99.8
$c_{11} + \frac{1}{2}c_{22}$	0.00927	0.01516	0.01404
Actual yield, bushels/acre	34.58	48.08	25.55
Optimum yield, bushels/acre	35.40	49.87	26.61
Loss of yield, bushels/acre	0.82	1.79	1.06‡
% loss of yield	2.37	3.72	4.17

Constants of equation given in Table 12.

In this experiment competition is primarily for water. The greater the plant density, the greater will be the amount of water lost by transpiration. This is recognized in Australia, where a bare fallow is found to be the best method of conserving soil moisture, and Sanders & Garner (unpublished) have collected data which show that the greater the number of sugar beet per acre the less the percentage moisture in the soil. Thus in those areas where plant density is high there will be an

increased loss of water, and the available water will be less than in areas of low plant density. Thus the small fraction extracted by the external plants from densely populated areas, negligible in the case of nutriments, is now appreciably due to the reduced total supply. For this reason the relative external competition for moisture in areas of high plant density is more intense than that for nutriments at a similar high plant density. This explains the high competition correlations at high plant density in Exp. A. The present work is not in sufficient detail to allow the verification of this hypothesis directly.

It has been argued by some workers that the irregularities of seeding found in farming practice in this country cause considerable loss of potential yield. The compensation due to propinquity in the cases observed is large enough to make the loss resulting from the uneven plant distribution very small. The loss of yield in the three experiments is 0.8, 1.8 and 1.1 bushels/acre respectively. This amounts to 2.4, 3.7 and 4.2 % of the crop in the three cases. The reduction appears to be typical of the loss of wheat in this country due to irregularities of plant density. Most of these irregularities are caused by the seed drill, and therefore there seems little need for more accurate drills. Uneven seed beds are also blamed for uneven plant density, but these experiments, carried out according to normal farm practice, indicate that present practice is adequate for wheat.

6. SUMMARY

An investigation of the problem of propinquity has shown that the yield of any unit of 6 in. of drill row of wheat is significantly affected by the number of plants in adjacent units. The "competition correlation", which measures the intensity of this effect, is greatest with medium plant densities, and low and often insignificant for units of high or low plant density.

Competition is primarily underground for water, soil nutriments and soil air; of these, competition for water is most important in determining yield.

The variation of plant number from unit to unit in a field, caused chiefly by irregularities of the seed drill, does not cause undue loss of yield. In three experiments 0.82, 1.79 and 1.06 bushels/acre (representing 2.37, 3.72 and 4.17 % of the optimum yield respectively) were lost owing to unevenness of plant.

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POPULATION STUDIES WITH WHEAT

III. SEED RATES IN NURSERY TRIALS AND FIELD PLOTS

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It has been shown that the yield from any area of wheat is influenced to some extent by the number of plants present. This, in turn, is determined by two factors—seed rate and percentage survival. The seed rate is determined by the setting of the seed drill, which can be altered at will, and the percentage survival by climate, disease, evenness of seed bed, bird damage, and viability of seed. Such causes are partly beyond the control of man: thus it is impossible to be sure of obtaining any given plant density in a field of wheat. This handicaps investigation of seed rates; indeed, the distinction between number of seeds sown, weight of seed sown, volume of seed sown and number of plant established is vague in much of the literature. In general, seed rate is tacitly assumed to be synonymous with plant density.

There are, therefore, two problems: first, to determine the optimum plant density, and secondly, to obtain that plant density in the field. This paper considers only the first of these two.

The investigations of Engledow and his co-workers (1925, 1926, 1928, 1930) have led to a detailed knowledge of the development of the wheat plant throughout its growth at various spatial intervals. They found that yield per acre is correlated with number of plants per acre, and argued from this that the areas of low plant density which occur in wheat fields cause a considerable loss of yield, and that higher seed rates and more even seeding should lead to greater returns. This work was based on data obtained from field plots. The conclusions were not confirmed by direct tests which Engledow & Ramiah (1930) carried out in nursery plots. The reasons for such discrepancies have been investigated by Fairfield Smith (1937). In this paper his methods have been amplified, and the divergence explained.

The discrepancies may be attributed to competition differences. In field plots each unit is, taking the average, subjected to the same degree of competition; in seed-rate trials in nursery plots each unit is surrounded by units of a similar density, so that low-plant densities have low competition, and high-plant densities have high competition. Thus in field trials areas of high-plant density have medium (or average) competition, whereas in

nursery trials they are subjected to high competition. Therefore the true yield of thick spacings in seed-rate trials should be *less* than that estimated from field trial data from the regression equation.

This may be illustrated by the data from the uniformity trial conducted at Cambridge in 1935-6 (Hudson, 1941). In investigating the propinquity effect, values were obtained individually for the mean plant number (*a*), the linear and curved regression (*b* and *c*) of plant number and yield for various intensities of competition. From these parameters a series of equations of the form

$$y = a + bx + cx^2,$$

can be formed for the various densities of competition, so that the relationship is given for *each* plant density of surrounding plot by a separate equation. Each equation will represent the regression of plant number on yield *with competition held constant*, and is thus directly comparable with regressions obtained from field trials. The correlations are all positive and significant, and the slope of the lines is similar to that obtained by Doughty & Engledow (1928) and Anon. (1926). They are shown in Fig. 1. This diagram also shows that for any plant density the less the intensity of competition the greater the yield.

On these graphs we can pick out *one* point on each line which gives the yield when plant number and the mean of the surrounding plant densities are the same. For example, in the competition class 30-33 it may be taken as the point corresponding to $32/4 = 8$ plants, and in the competition class 54-57 the point corresponding to 14 plants. The line drawn through these points gives a graph analogous to that obtained from seed-rate trials in nursery plots and shows the true relationship between plant density and yield. Fig. 1 shows that, whereas the optimum plant density appears to be over 20 plants per unit from the field data, the true optimum is about 9 plants per unit.

These graphs show that the intensity of external competition affects the yield. Clements *et al.* (1929) have shown that competition is primarily for water and secondarily for soil nutrient. Therefore the effect of competition will be more or less intense according to the amount of water and soil nutrient present. The degree of intensity of this competition will, along with other factors, decide the optimum plant density. This is best demonstrated by considering the cases of very great and very small competition intensities. If there is no competition the lines in Fig. 1 would be coincident, and the optimum plant density would be high and the same as that predicted by field studies. If competition was

intense the lines would be wide apart, as in the graphs shown in Fig. 1. Due to the large curvature of the line for low competition and the smaller curvature for the line for high competition the optimum plant density is low, and the curvature of the line marked "seed rate" (indicative of the importance of plant density in determining yield) is large.

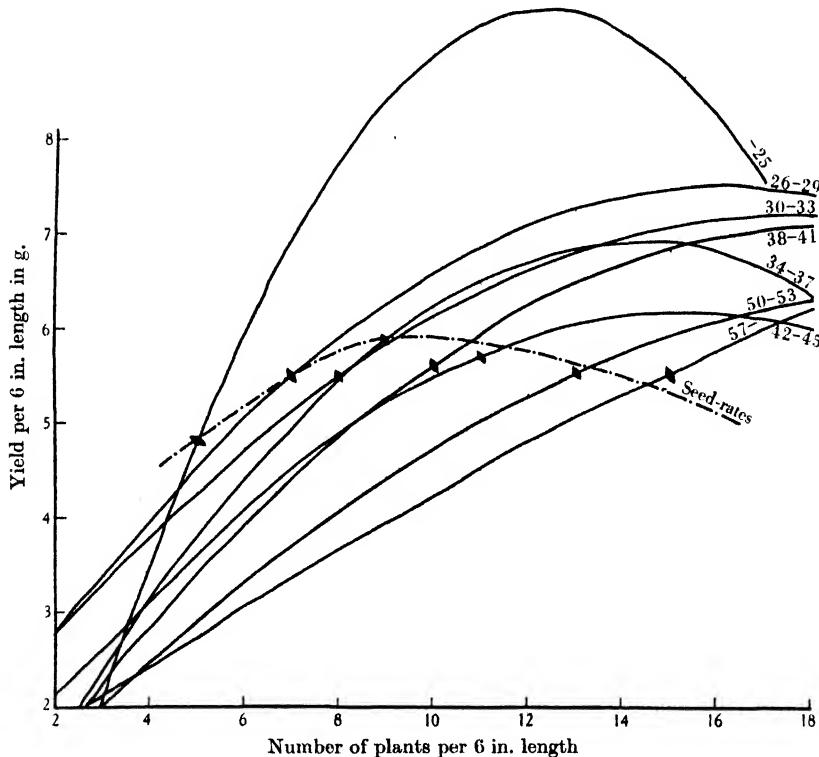


Fig. 1. To show the yield at various plant densities for varying degrees of competition. The figure at the right hand edge of each line shows the competition class which that line represents.

Since competition is primarily for water and secondarily for soil nutrient, the drier the climate and the poorer the soil the more intense will competition be, and the lower will be the optimum seed rate. A survey of the literature concerning seed rates confirms this (see Table 1). In countries where the climate is dry, such as U.S.A. and Australia, low seed rates of $\frac{1}{2}$ - $1\frac{1}{2}$ bushels per acre are found to be optimum; whereas in the moister climate of England considerably higher

Table 1. *Summary of literature concerning the optimum seed rate of winter wheat. Seed rates in amount per acre*

Paper by U.S.A.	Locality	Optimum seed rate	Remarks
Hickman, M. S.	Ohio, 1890	7 pecks	Average of 9 years, little difference, 4-8 pecks
Hunt, I. F.	Illinois, 1890	4, 5 and 8 pecks	Range 3-8 pecks
Latta, W. C.	Indiana, 1890-1900	Highest	Range 2-8 pecks
Geordeson, C. C. <i>et al.</i>	Kansas, 1892	8 pecks	Yields 30-38 bushels per annum, little difference, 5-8 pecks
Morrow, G. E. & Gardner, F. D.	Illinois, 1892	No real difference	Range 4-8 pecks, yield 24-28 bushels per annum
Hays, W. M.	North Dakota, 1893	5½ pecks	Range 2-6 pecks
Morrow, G. G. & Gardner, F. D.	Illinois, 1894	4-8 pecks	—
Morrow, G. E. & Bone, J. H.	Oklahoma, 1896	6 pecks	Range 3-8 pecks
Mackay, A.	Canada, 1896	1½ bushels	—
Hickman, J. F.	Ohio, 1897	10 pecks	Adverse conditions
Zavitz, C. A.	Ontario, 1897	1½ or 2 bushels	5 years' work
Sheppherd, J. H. & Ten Eych, A. M.	Oklahoma, 1898	8 pecks	Range 3-8 pecks
Snyder, W. P. & Burr, W. W.	North Dakota, 1900	5½ pecks	4 years' averages
Merrill, L. A.	Nebraska, 1909	2, 4 or 5 pecks	55 bushel crop
Williams, C. G. & Welton, F. A.	Utah, 1910	3 pecks	Confirms local prac- tice
Schollander, E. G.	Ohio, 1911	8 pecks	Average 14 seasons
Ross, J. F. & Leidigh, A. H.	Willister (North Dakota), 1912	5 pecks	Range 3-7 pecks
Montgomery, E. G.	Tennessee, 1912	3 pecks	Yield 20 bushels per annum
McMurdo, G. A.	Nebraska, 1912	48 plants per foot	Maximum density best
Jardine, W. M.	Colorado, 1916	3 pecks	—
Stephen, D. E. & Hilt, C. E.	Kansas, 1916	8 pecks	Range 2-8 pecks
Nole, C. F.	Oregon, 1917	Early sowing, 8 pecks	20 bushels per an- num
Steward, R. L.	Pennsylvania, 1917	Late sowing, 3 pecks	13 bushels per an- num
Bergh, O. I. <i>et al.</i>	New Mexico, 1918	6 pecks	Range 2-8 pecks
Bracken, A. F. <i>et al.</i>	Minnesota, 1919	Irrigated 4-6 pecks	—
	1923	Non-irrigated 2½-3½ pecks	—
	Idaho, 1924	6 pecks	Minimum, dry land
	Utah, 1925	4-5 pecks	—
	Kansas, 1928	4 pecks	No difference dry and irrigated land
		5 pecks and over	Heavier in dry au- tumn
		4 pecks	Yield 7-12 bushels per annum
		2-4 pecks	—

Table 1 (cont.)

Paper by	Locality	Optimum seed rate	Remarks
Bracken, A. F. & Stewart, G.	Utah, 1930	5-6 pecks	Lighter seeding advised on silts and sandy loam
Leighty, C. E. & Taylor, J. W.	U.S.D.A. 1927	6 pecks	Range 2-8 pecks
Hutchison, R. G.	Oregon, 1936	96-129 lb.	Irrigated
Godel, G. E.	Canada, 1935	1½-2½ bushels	—
Great Britain			
Dowling, R. N.	Wye, 1908	4 bushels or over	Range ½-4 bushels
Engledow, F. L.	Cambridge, 1925	Red Fyfe 4-24 plants. Hybrid 4 plants	Plants per foot
Harrison, R. M.	Wye, 1932	5-7 bushels same as 2-7, both better than 1-2 bushels	—
Australia			
Forster, H. C. & Vasey, A. J.	Victoria, Australia, 1930	45 lb. best, little difference 45-120 lb.	Range 45-180 lb.
Macmillan, J. R. A.	New South Wales, 1937	3-8-16 plants	Plants per foot, range 3-8-64
Sutton, G. L.	New South Wales, 1911	57 and 36 lb.	Both 22 lb.
McDiarmid, R. W.	New South Wales, 1911	45-60 lb.	Both 30 lb.
Downing, R. G.	New South Wales, 1911	40 lb.	30 and 20 lb.
New Zealand			
Frenkel, O. H.	New Zealand, 1930	(a) 3-4 plants (b) 11-27 plants (c) 3-4 plants	Small differences, plants per foot

seed rates should be used. Where land is fertile high seed rates are found to be necessary, and in the few experiments with irrigated wheat they are higher than usual. The majority of papers reviewed also show that the variation of yield caused by sowing seed at rates other than the optimum is surprisingly small; a variation of 1-2 bushels per acre frequently causes very little variation of yield.

It is impossible to forecast the percentage survival in any field with accuracy, and difficult to foresee whether competition for moisture is likely to be severe. For this reason it is perhaps fortunate that considerable deviation from the optimum seed rate does not influence the yield very greatly. It is, however, possible to use these principles to some extent in practical advice. For example, the moisture content of heavy land will be greater than that of light land, and thus the competition for water should be less and the optimum seed rate higher. Fields liable to flooding are liable to be short of soil air, which in certain

circumstances may be limiting. In such cases competition for soil air will occur, and the field should be sown sparingly. Also soils in a high state of fertility should have more seed than poor soils. These, however, are all secondary considerations which cause the optimum to fluctuate around optima dictated by the climatic conditions.

SUMMARY

The discrepancies between nursery plots and field plots, found by previous workers when investigating yield problems with wheat, are discussed. The reason for these differences is given, and the true optimum plant density identified.

It is shown that the intensity of competition for water and soil nutriments influences the optimum plant density, and that a high intensity of competition is associated with a low optimum plant density. A summary of literature is given, and the varying values for the optimum seed rates in different parts of the world interpreted in the light of water and soil nutrient supplies.

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STUDIES ON THE EXCRETION OF COPPER IN THE RABBIT

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(With Two Text-figures)

COMPARATIVELY few studies have been made of the channels of elimination of copper in the animal body following parenteral and intravenous administration of the element. Lindow *et al.* (1929), in studying the excretion of copper in rats, found that with a normal diet about two-thirds were excreted in the faeces and one-third in the urine. When this diet was supplemented with copper, 98% of the supplement was excreted in the faeces although the urinary concentration was increased five-fold. Cunningham (1931) found that rats on a low copper diet excreted about three-quarters of the total in the faeces and one-quarter in the urine. The copper content of normal human urine, according to Rabinowitch (1933), varies from traces to 0.7 mg. for a 24 hr. period, with an average excretion of 0.41 mg. per litre, whilst Ross & Rabinowitch (1935) found the urine of children to contain, on an average, 0.3 mg. per litre. Tompsett (1934) gave the limits of human urine as 0.08-0.48 mg. per litre, and further cited data on copper intake and excretion in seventeen observations on the human subject. Analysis of his data shows that the average daily food intake of these patients (under hospital dietary regime) was 2.11 mg., the faecal output being 1.77 mg. and that of the urine 0.32 mg., a figure of a similar order to that reported by Rabinowitch. In one patient suffering from carcinoma of the stomach and receiving a fluid diet containing only 0.21 mg. daily, the faecal excretion was 0.52 mg. and that of the urine 0.11 mg. per day. The relationship of faecal to urinary copper, however, can have little meaning in itself and must depend upon the content of available copper in the food. Ingestion of large amounts of copper in very insoluble form would enormously increase the faecal output without materially altering the urinary figure.

The rapid disappearance from the blood stream of abnormal quantities of copper, intravenously injected, has already been reported (Eden & Green, 1939), and further, the continuous ingestion of copper has been

shown to have comparatively little effect on the blood copper content until a definite pathological condition has been set up (Eden, 1940a). The present paper deals with the elimination of abnormal quantities of copper introduced by various routes into rabbits. This species was selected for laboratory metabolism trials since the amount of excreta is small, while, by a specially designed metabolism cage and collecting apparatus, it was possible to obtain automatic separation of urine and faecal pellets; the liquid urine running down the sloping sides into an outer funnel and the firm pellets rebounding into the mouth of a second funnel set within at a spacing less than pellet diameter. At the time these experiments were commenced, the peculiar habit of "physiological faecal refection" by the rabbit was not generally known, and some of the tentative conclusions originally reached about the apparently peculiar behaviour of copper have had to be modified.

A knowledge of the habits of individual rabbits was obtained during a preliminary period and helped considerably in the collection of the separate excreta, especially when, after injection or oral dosing with copper, a closer watch was kept to ensure that the faeces and urine were obtained without mutual contamination. For example, certain rabbits were observed to pass a whole day's urine at a single micturition. All the rabbits in these trials weighed about 2 kg. and were fed on a mixture of equal parts by weight of bran and oats, the amount of food given, 40 g., being such that it was comfortably cleared up each day, so obviating analysis of residues for copper. The feeding of green food was omitted, as this varied according to the season and would also have involved daily analyses for copper. Water, however, was always freely available.

The procedure adopted throughout was as follows. The rabbit was given the experimental ration but no excreta were weighed or measured for 3 days, during which the animal was getting accustomed to the experimental conditions. After this period the daily collection of urine and faeces commenced. Each morning the day's ration was weighed out and moistened with a little water. The urine passed was measured and aliquots taken for analysis; if the amount was scanty two or more days' samples were mixed. The faeces were mixed in a mortar after being weighed and samples weighed out for copper analysis and dry-matter determinations. Following injection or oral dosing the rabbit would sometimes go off feed for a day and the subsequent day's food was then added to the residues; this was all cleared up within a day or so.

As an expression of the rate of copper excretion in the faeces, it was

decided that owing to natural variations in the daily amounts and moisture content of faeces this could best be shown as mg. copper per 100 g. faecal dry matter. On constant diet the theoretical output of faecal dry matter is also constant so that the concentration of copper in the dry matter shows the true rate of passage into the rectum of copper taken in by the mouth, irrespective of the regularity of evacuation from day to day.

Table 1 gives the full daily data obtained with rabbit C, receiving 20 g. each of bran and oats daily, of copper content 0.298 mg., and illustrates the procedure adopted throughout the trials.

Table 1. *Metabolism trial I: rabbit C*

Day	Copper intake	Faeces				Urine		
		Weight	Dry matter	Copper per	Total copper	Vol.	Copper per	Total copper
				100 g. dry matter			c.c.	
1	0.298	21.2	50.3	2.58	0.276	40	0.31	0.012
2	0.298	13.0	48.9	2.31	0.150	43	0.37	0.016
3	0.298	20.7	53.2	2.20	0.242	52	0.29	0.015
4	0.298	25.7	50.4	2.30	0.298	34	0.20	0.007
5	0.298	27.5	49.2	2.46	0.333	48	0.38	0.018
6	0.298	25.1	48.6	2.43	0.296	40	0.28	0.011
7	0.298	36.6	48.1	2.33	0.410	37	0.18	0.007
8	0.298	19.9	51.9	2.24	0.231	40	0.18	0.007
9	0.298	13.0	48.3	2.46	0.155	40	0.32	0.013

Total copper intake by food = 2.682 mg.

Copper excreted in faeces = 2.391 mg.

Copper excreted in urine = 0.106 mg.

Total excretion = 2.497 mg.

Average daily in food = 0.298 mg.

Average daily in faeces = 0.266 mg.

Average daily in urine = 0.012 mg.

In all the metabolism trials conducted in this series a preliminary period was given during which data were obtained for the normal excretion. At this point it is convenient to summarize these preliminary data without reference to the actual experiments which followed (Table 2).

Table 2. *Copper metabolism experiments with rabbits on standard diets*

Reference	Rabbit no.	Duration of period days	Copper intake mg.	Copper excreted in faeces mg.	Copper excreted in urine mg.	Total copper excreted mg.	Ratio of faecal to urinary copper
Trial I	C	9	0.298	0.266	0.012	0.278	24.1
II	E	11	0.557	0.547	0.012	0.559	45.6
III	A	9	0.513	0.494	0.012	0.506	41.2
IV	D	8	0.298	0.266	0.011	0.277	24.1
V	B	8	0.400	0.347	0.006	0.353	57.8
VI	D	10	0.306	0.300	0.012	0.312	25.0
VII	D	7	0.300	0.260	0.015	0.275	17.3

The results given in Table 2 demonstrate the obvious expectation that adult rabbits on normal diet are in approximate copper equilibrium, intake with the food being balanced by output in the excreta, and incidentally establish the accuracy of the experimental technique. By far the greater elimination occurs through the faeces, agreeing with the findings of Tompsett (1934) in his studies of the copper excretion of humans receiving a mixed diet. On the particular diet employed, about 96% of the total copper was excreted through the faeces in the rabbit, compared with 84% for human beings and 66-75% recorded for the rat. The copper content of rabbit urine under the standard conditions employed lay between 0.07 and 0.38 mg. per litre, being of about the order reported for human urine by Rabinowitch (1933) and by Tompsett (1934).

EXCRETION OF COPPER ADMINISTERED INTRAVENOUSLY

The fate of intravenously injected copper was studied with rabbit D in trial IV, details of the preliminary period of which have been given in Table 2. The preliminary period lasted 8 days, and then 3 mg. copper as the sulphate contained in 1 ml. solution were injected into the ear. In Table 3 are quoted the relevant data for the 2 days preceding and the 7 days subsequent to the injection.

Table 3. *Rabbit D: 3 mg. copper intravenously injected on the 8th day*

Day	Copper in food	Faeces			Urine		
		Total weight g.	Copper per 100 g. dry matter mg.	Copper excreted mg.	Vol. c.c.	Copper per litre mg.	Copper excreted mg.
7	0.298	32.3	2.48	0.329	60	0.21	0.013
8	0.298	35.2	2.75	0.359	35	0.15	0.005
9	0.298	22.0	4.50	0.359	82	5.24	0.430
10	0.298	12.9	3.43	0.245	43	2.16	0.093
11	0.298	32.0	3.66	0.502	77	0.32	0.025
12	0.298	22.3	4.03	0.428	41	0.95	0.039
13	0.298	27.8	4.57	0.623	34	0.20	0.007
14	0.298	35.0	4.26	0.738	67	0.15	0.010
15	0.298	21.9	3.95	0.431	25	0.20	0.005

Over the preliminary period of 7 days the urinary copper had varied from 0.15 to 0.34 mg. per litre, but in the 24 hr. following the injection the copper content rose to 5.24 mg. per litre. There was a rapid fall over the two succeeding days, a slight rise again on the fourth day after injection, and then a fall to the original normal level which was maintained for the remainder of the experiment. By far the major portion

of the urinary excretion occurs within the first 24 hr., and this is readily correlated to the general blood picture under such conditions (Eden & Green, 1939). While the blood level is above normal, the kidneys play a prominent part in elimination of copper from the circulation, but once the normal level is regained (certainly within 24 hr.), the kidneys have little further role in the elimination of residual quantities of the metal stored in the tissues. During the 7 days following injection, about 0.61 mg. copper was eliminated in the urine compared with the preceding week's figure of about 0.07 mg. Thus, of 3 mg. injected, only 0.54 mg., or 18%, was eliminated in the urine, of which 14% was eliminated during the first 24 hr. and a further 3% by the end of the second day.

The faecal output of copper, best shown by the concentration in the faecal dry matter, rose following the injection, by excretion of copper from the circulation through the bile and unidentified areas of the intestinal wall. This rise was maintained over the next 8 days, after which there was a gradual fall to the normal level.

The relative rise in the concentration of copper in the faeces is not so pronounced as that in the urine; on the other hand, return to normal is very much more gradual, not being complete until the fourth week. The possible significance and explanation of this slow return to normality is deferred until the discussion.

In Table 4 are given the summarized data of the weekly eliminations of copper in urine and faeces of the same rabbit.

Table 4. *Summarized data of metabolism trial IV. Rabbit D: intravenous injection of 3 mg. copper*

Period	Duration days	Copper intake mg.	Copper excreted		Daily averages		
			in faeces mg.	in urine mg.	Food mg.	Faeces mg.	Urine mg.
Preliminary	8	2.38	2.12	0.089	0.298	0.286	0.012
(Injection of 3 mg. copper)							
1st week	7	2.00	3.36	0.608	0.298	0.475	0.087
2nd week	7	2.09	2.64	0.097	0.298	0.377	0.014
3rd week	7	2.09	2.41	0.066	0.298	0.344	0.010
4th week	7	2.09	2.36	0.101	0.298	0.338	0.014
Total	36	10.74	12.89	0.961			

It will be noted that the greater part of the injected copper is eliminated during the first week, the excess of the metal in the excreta above that in the food being about 1.85 mg. or nearly 62% of the quantity injected. Of this, 0.54 mg., or 18%, was excreted in the urine and 44% in the faeces, showing that the intestine is the channel of elimination of the greater part of copper introduced directly into the

blood stream. During the succeeding weeks, urinary excretion was normal. An excess of faecal above food copper of 0.65 mg., or 22% of the amount injected, was found during the second week, 0.38 mg. (13%) during the third week, and the remaining 3% during the fourth week, at the end of which the copper figure on the dry-matter basis had fallen practically to normal. Over the 36 days of experiment 10.73 mg. copper had been taken in with the food and 3 mg. by injection, whilst 13.82 mg. had been recovered in the excreta, 12.86 mg. being in the faeces. Thus, within the limits of experimental error, all the copper was accounted for. It is remarkable that despite the introduction of highly ionized copper directly into the blood stream the amount eliminated in the urine was only some 18%. The faeces, the principal channel of elimination of food copper, are also the main route of elimination of intravenously injected copper.

ELIMINATION OF COPPER FOLLOWING DOSING BY STOMACH TUBE

Rabbit D was later employed in trial VI, in a study of the paths of elimination of copper from the body following administration in aqueous solution by stomach tube. After a preliminary period of 10 days on the usual ration, 50 mg. copper as the sulphate in 5 ml. solution were introduced from a syringe through a greased catheter into the stomach of the rabbit and rinsed through with a little water. The animal was off feed for a day following dosing, but residues were cleared with the second day's food. In Table 5 are given complete data, for the 2 days preceding and for 7 days subsequent to the dosing.

Table 5. *Rabbit D: excretion of copper following stomach tube administration of 50 mg. copper. Dosing on day 10*

Day	Copper in food mg.	Faeces			Urine		
		Total weight g.	Copper per 100 g. dry matter mg.	Copper excreted mg.	Vol. c.c.	Copper per litre mg.	Copper excreted mg.
9	0.306	36.2	2.29	0.36	55	0.18	0.010
10	0.306	23.2	2.14	0.24	58	0.11	0.006
11	0.306	26.2	5.04	0.67	110	4.18	0.459
12	0.306	12.7	64.99	4.12	77	0.35	0.027
13	0.306	19.1	67.02	6.86	54	0.20	0.011
14	0.306	25.5	53.22	6.38	70	0.18	0.013
15	0.306	29.5	46.66	6.30	104	0.10	0.010
16	0.306	20.7	40.09	4.33	118	0.15	0.018
17	0.306	29.2	33.88	4.20	100	0.22	0.022

The urinary copper in the preliminary period ranged from 0.09 to 0.19 mg. per litre, but in the 24 hr. following dosing with 50 mg. copper

there was a rise to a figure of 4.18 mg. per litre, corresponding to an absolute excretion of 0.46 mg. During the second day this figure fell to a value less than twice the previous average, and on the third and subsequent days the copper concentration in the urine was normal. The rise and fall of blood copper following dosing of copper by stomach tube (Eden & Green, 1939) is such that the blood level becomes normal again within 24 hr., and the present experiment shows that it is only during this period that the urinary copper is significantly elevated above its normal range.

The faecal excretion expressed on the percentage dry-matter basis was doubled in the first 24 hr. after dosing. During the second day it rose to about thirty times the normal level and remained high, still being fifteen times the normal at the end of a week, in contrast to the sudden drop in the urinary figure on the second day.

The excretion was followed for 29 days after dosing, and in Table 6 are reported the summarized data for the preliminary period and for each of the 4 weeks subsequent to the administration of copper.

Table 6. *Summarized data of trial VI. Rabbit D: administration of 50 mg. copper by stomach tube*

Period	Duration days	Copper intake by food mg.	Copper excreted in faeces mg.	Copper excreted in urine mg.	Daily averages		
					Food mg.	Faeces mg.	Urine mg.
Preliminary	10	3.06	3.00	0.12	0.306	0.300	0.012
(Dosing with 50 mg. copper)							
1st week	7	2.14	32.88	0.56	0.306	4.70	0.080
2nd week	7	2.14	13.79	0.09	0.306	1.97	0.012
3rd week	7	2.14	3.84	0.08	0.306	0.55	0.011
4th week	8	2.45	2.36	0.07	0.306	0.30	0.009
Total	39	11.93	55.87	0.92			

By comparison with the daily averages for the preliminary period it will be observed that during the first week only 0.56 mg. less 0.09 mg., or about 0.47 mg., i.e. roughly 1% of the total dosage, appears in the urine. The actual quantity of copper recovered above that found in the food was about 45 mg. of the supposedly 50 mg. given, but the difference may well be due to a possible loss of a small quantity of the original 5 ml. solution in administration through the catheter, plus a summation of errors involved in the subsequent twenty-nine daily analyses and samplings. During the last 8 days the animal was practically in copper equilibrium. Taking the actual amount recovered, 45 mg., as a basis for calculation, about 30.8 mg. or 69%, were recovered from the faeces

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over the first week with a total recovery of 70% when the urinary excretion is included.

During the next week another 11.6 mg. copper were recovered in the faeces, while the remainder was eliminated during the third week.

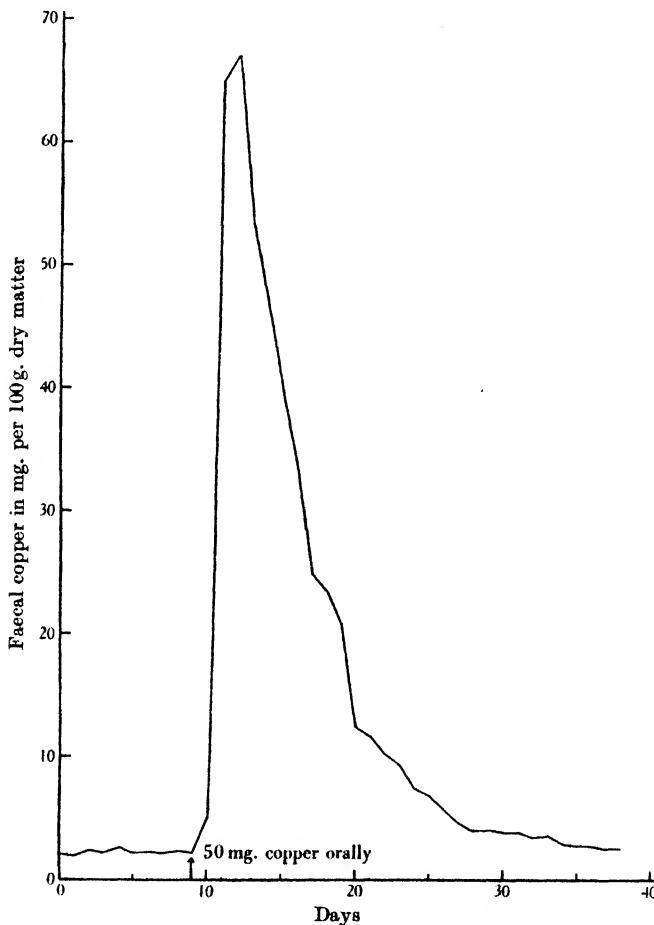


Fig. 1. Rate of excretion of copper in faeces following administration of 50 mg. copper (as the sulphate) by stomach tube to rabbit D.

The rate of elimination of the copper, expressed on the percentage dry-matter basis to eliminate daily variation in times of defaecation, is shown graphically in Fig. 1; the urinary excretion being omitted because, apart from the first day after dosing, the excretion was maintained at normal levels throughout the experiment.

**ELIMINATION OF COPPER FOLLOWING INCORPORATION OF THE
ELEMENT ON TO THE FOOD**

The final trial (VII) in this series was again made with rabbit D. After a preliminary period of a week, 50 mg. copper as the sulphate in solution were incorporated in the normal ration of bran and oats and *dried* on to the food by heating at 100° C. in an oven for a short time. This was then fed to the animal the following day and the rate of elimination in the excreta again studied. In this trial the daily samples of urine were mixed, preserved with a drop of chloroform, kept in the refrigerator, and analysed weekly except for the 2 days subsequent to the feeding of the copperized ration.

The rabbit ate well for the following month, but during the fifth week it went badly off feed, becoming dull and listless, and, since by this time the copper content of the faeces had almost attained its normal value, it was decided to terminate the experiment. As before, the full daily data for the 2 days preceding and the 7 days subsequent to the feeding of copper are given in Table 7.

*Table 7. Rabbit D. Excretion of copper following administration
of 50 mg. copper dried on to the food given on day 7*

Day	Faeces				Urine		
	Copper in food mg.	Total weight g.	Copper per 100 g. dry matter mg.	Copper excreted mg.	Vol. c.c.	Copper per litre mg.	Copper excreted mg.
6	0.300	28.4	2.82	0.37	38	0.17	0.006
7	0.300	23.1	2.88	0.33	46		0.007
8	0.300	9.9	12.77	0.53	195	0.56	0.110
9	0.300	19.2	43.54	4.20	105	0.18	0.019
10	0.300	20.0	46.37	3.06	150		
11	0.300	31.3	43.27	6.45	60		
12	0.300	17.0	33.33	3.31	90	0.16	0.061
13	0.300	20.1	33.35	2.93	35		
14	0.300	28.1	37.52	3.93	60		

It is seen that there was a slight rise in the urinary excretion, much less than in the case of administration of soluble copper by stomach tube, on the day following the feeding of the copper. On the second day this excretion had fallen to the normal level and was maintained at such for the remainder of the trial. The faecal output, expressed on the percentage dry-matter basis, rose abruptly after feeding to a maximum on the third day, remaining high and only slowly falling as indicated graphically in Fig. 2.

In Table 8 are given the summarized data of the copper intake and excretion for the preliminary period and for each of the subsequent 5 weeks following the ingestion of the high quantity of copper.

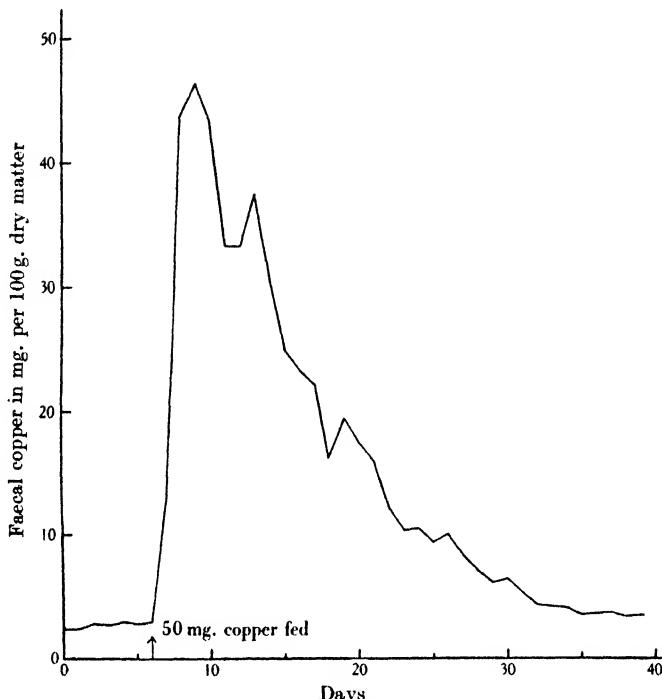


Fig. 2. Rate of excretion of copper following feeding of 50 mg. copper dried on to food.

Table 8. *Summarized data of metabolism trial VII. Rabbit D: administration of 50 mg. copper dried on to the food*

Period	Duration days	Copper intake by food mg.	Copper excreted in faeces mg.	Copper excreted in urine mg.	Daily averages		
					Food mg.	Faeces mg.	Urine mg.
Preliminary	7	2.10	1.82	0.104	0.300	0.26	0.015
(50 mg. copper fed)							
1st week	7	2.10	24.42	0.191	0.300	3.49	0.027
2nd week	7	2.10	15.92	0.085	0.300	2.28	0.012
3rd week	7	2.10	8.46	0.103	0.300	1.21	0.015
4th week	7	2.10	4.40	0.064	0.300	0.63	0.009
5th week	5	1.50	2.75	0.071	0.300	0.55	0.014
Total	40	12.0	57.77	0.618			

These data show that the urinary output remained normal throughout the whole experiment with, as already mentioned, the exception of the

day following feeding of the copperized ration. During the first week after copper feeding about 22.2 mg. were excreted in the faeces in excess of intake, and about 0.1 mg. above normal in the urine, with a progressive diminution over the subsequent weeks. The total amount of copper excreted in the course of this trial was 58.4 mg., of which 12 mg. had been taken in by food. Thus 46.4 of the 50 mg. fed were accounted for, and the faecal excretion was still above normal over the final days. Of this amount less than 0.1 mg. appeared in the urine, that is, only about 0.2%. During the first week 47.7% of the 46.4 mg. recovered appeared in the faeces, 30% during the second, 14% in the third and 5.1% in the fourth week. 97% of the total copper eliminated appeared within a month, the rest during the last 5 days.

Thus, although the elimination of copper was slower in this experiment than when given in solution by stomach tube, less was actually absorbed into the body as reflected by the almost insignificant absolute amounts appearing in the urine. This small absorption into the body of copper *dried on* to the food explains why chronic copper poisoning is difficult to induce (Eden, 1940), although even under these conditions there is some storage of copper in the liver when feeding is prolonged. A quantity of 100 or 200 mg. copper, which is rapidly fatal when given as a single dose by stomach tube, can be tolerated for months when fed dried on to the food (Eden & Green, 1939).

EXPLANATION OF PROLONGED DELAY IN FAECAL EXCRETION

Although the faeces play the greater part in elimination of copper, the long period necessary for complete excretion is noteworthy and at first sight very surprising. Periods of from 3 to over 5 weeks were required before recovery in faeces was completely accomplished, and although in the case of intravenously injected copper one might have postulated a possible fixation by the tissues and a subsequent slow release, in the cases of the 50 mg. dosed or fed dried on to the diet there was every reason for believing that by far the greater part of the copper never left the alimentary tract at all. The small amounts excreted in the urine and stored in the tissues, and the absence of any toxic symptoms are all evidence of the slight degree of absorption into the blood stream. There would appear to be an exceedingly low kidney threshold value for copper, any significant rise in the blood copper content causing part of the excess copper to appear in the urine; thus the urine is a significant channel of elimination of copper from the body only when elevated blood

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copper levels are encountered, and absence of urinary elimination is an indication of absence of significant rise in blood level.

One remote possibility was that, since the metal had not been absorbed into the body nor been recovered in the faeces during the first few days, it was retained in some way on the wall of the tract, to be eliminated by a process of exfoliation of the cells. Failing that, the only logical conclusion that could meet the facts was the postulation of some kind of copper cycle, whereby copper was returned to the contents of the tract.

A rabbit was again fed 50 mg. copper dried on to the food and the excreta collected for a week, after which the animal was killed and the alimentary tract and contents as well as the major soft tissues removed for analysis. Of the 50 mg. copper fed about 38 mg. were recovered in the week's faeces in excess of the food intake. The tissues and wall of the tract contained only the normal 0.2-0.5 mg. % fresh material, but the copper contents of different portions of the tract were stomach 2.5 mg., intestines 1 mg., caecum 5 mg., and rectum 3.5 mg. Thus the whole of the copper fed was accounted for, and the quantity not recovered in the excreta was found in the contents of the tract.

About this time, Madsen (1939), reviving an obscure publication of Morot's in 1882, showed that the rabbit normally passes two kinds of faeces, one the ordinary pellet "day" type and the other a soft mucous type passed during the night. This latter type is rarely seen under ordinary conditions, since the rabbit collects them directly from its anus and swallows them. Taylor (1939) confirmed the observation and subsequently (1940) enlarged upon it. Eden (1940b), repeating the experiment of feeding 50 mg. copper dried on to the food to a rabbit, with and without a wooden collar large enough to prevent the animal getting at its anus, showed that this process of "physiological faecal refection" was of very high magnitude. By observations not only of the copper output but of the amount of daily dry matter passed, with intermittent collaring of the rabbit, it was shown that from about 50 to 90 % of the dry matter of faeces passing the anus each day was reconsumed. Since the amount of dry matter of the "night" faeces is only about half of that passed in the "day" faeces, the reconsumption of night faeces alone represents a level of refection of only 33 %, so that some "day" faeces must in addition have been reconsumed. Close observation of the rabbit showed that dry pellets are taken from the anus during the day and swallowed after chewing, in contrast to the night faeces which are believed to be swallowed whole. Under conditions of collaring, 10 mg. copper were excreted the first day after feeding,

25.5 mg. the second, 6.9 and 5.7 mg. on the third and fourth days respectively, and the remaining 2 mg. were excreted within the next 2 or 3 days. This 96% elimination of ingested copper in 5 days when faecal refection was mechanically prevented is much more in line with normal expectation of rate of passage of food along the tract. It may be taken as the "real rate" of copper excretion, in contrast to the "apparent rate" created by the balance between faeces actually passed and faeces reconsumed. The "apparent excretion" of the same rabbit uncollared happened to be 38 mg. or 76% in 7 days, but this is comparatively high, as shown by rabbit D in trial VII, from which 22.5 mg. or only 45% were recaptured in 7 days. Individual rabbits can apparently vary considerably in the extent of faecal refection, and hence in the difference between "apparent rate" of excretion of copper and "real rate" when refection is excluded.

Thus what one might initially have assumed as a peculiar attribute of the element copper was shown to be due to the more far-reaching and important, though unsuspected, physiological habits of the experimental animal selected. Copper in this case, especially when dried on to the food, really acted as a kind of "food marker" such as *Lycopodium* spores or charcoal, which have to be assessed by microscopic methods and which are markers more in the qualitative than in the quantitative sense.

The low absorption of copper into the body may be partly an attribute of the copper ion itself, but since flushing the tract by stomach tube, with soluble copper in dilute solution, results in much higher absorption, the behaviour is best explained by formation of insoluble copper compounds by interaction with decomposition products in the tract.

Although from the length of time required for complete elimination of copper from the alimentary tract of the rabbit some portions of the original material fed must undergo a number of successive cycles from anus to mouth, the copper itself must actually be in very insoluble form, since the urinary figures supply no evidence of any detectable absorption after the initial phase. Whatever may be the nature of this copper compound it is not unlikely that it is a sulphur derivative, such as copper sulphide, produced by the interaction of copper with sulphuretted hydrogen or related substances, formed by bacterial fermentation in the lower portion of the tract. The reswallowing of faeces containing such sulphur derivatives would tend to render insoluble any soluble copper already in the stomach, and it is possible that such detoxication may explain the great difficulty in inducing chronic copper poisoning in the rabbit as already reported (Eden, 1940a).

Though this work was undertaken to throw further light on the physiology of copper and indeed establishes the fact that the faeces are the principal channel of elimination of the element from the animal body however the copper is introduced, the choice of the rabbit for metabolism studies in the laboratory has incidentally been fortunate in that it helped in acquiring facts concerning the fundamental physiology of this species.

SUMMARY

1. In metabolism experiments undertaken to study the channels of elimination of copper under varying conditions, the rabbit was chosen as experimental animal for convenience of manipulation. This choice, although not defeating the main objects of the work, accidentally added valuable information on the little-known habit of "physiological faecal refection" in this animal.

2. On a normal diet of bran and oats, containing the usual traces of copper, the rabbit is in copper equilibrium, excreting as much in faeces and urine as is taken in the food. Urinary elimination is of the order of 0.07-0.38 mg. per litre, but this is very subsidiary to faecal elimination of about 2.5 mg. per 100 g. of dry matter, which may account for even more than 96 % of the total food copper.

3. Increase of food copper is not reflected by material increase of urinary output, the higher quantities appearing almost entirely in the faeces. Of 50 mg. of copper or nearly 200 times the normal food intake, as the dissolved sulphate dried on to the food, only 0.1 mg. appeared in the urine, the remainder being slowly excreted in the faeces over the unexpectedly long period of 4 or 5 weeks.

4. The same quantity of copper, 50 mg. as the sulphate, given in dilute solution by stomach tube and so flushing the alimentary tract, resulted in a rise in urinary output returning to normal within 48 hr. The quantity so eliminated was, however, only 0.47 mg. or less than 1 % of the total, the remainder being gradually recovered in the faeces collected over the ensuing 3 weeks.

5. Following intravenous injection the copper content of the urine rose sharply but returned to normal within a day or two. The renal threshold is apparently low, and increased urinary output can be taken as the index of a period of temporarily raised blood copper. The faecal copper simultaneously rose, but its return to normal was spread over 4 weeks. Of 3 mg. of copper injected as the sulphate into an ear vein, only 0.53 mg., or 18 %, appeared in the urine, and of this 17 % appeared

within 48 hr. The remaining 82% appeared in the faeces, 44% in the first week, 22% in the second, 13% in the third, and 3% in the fourth. Hence, whatever be the channel of entry into the body the main elimination takes the faecal route.

6. Assuming absorption of ingested copper by the alimentary tract to be low, and elimination of injected copper to occur through the bile and unidentified areas of the intestinal wall, the recorded results presented no difficulty in interpretation except for the unexpected delay in faecal excretion extending over several weeks.

Analysis of a rabbit fed on copperized food and killed after 7 days' collection of faeces showed only the normal traces of copper in the various soft tissues, and the whole of the 24% of unrecovered copper present in the actual contents of the alimentary tract, 5% being present in the stomach. The forgotten observations of Morot in 1882, revived by Madsen in 1939, then provided the clue, and the data found a ready explanation on the basis of "normal physiological faecal refection" peculiar to the rabbit. When fitted with a wooden collar to prevent access of mouthparts to anus the "true rate" of excretion of copper was revealed as in line with expectation in regard to normal rate of food passage through the tract, the same rabbit now excreting 96% of the ingested copper in the faeces of the first 5 days.

7. Ordinary healthy laboratory rabbits habitually practise "faecal refection" on an extensive scale, from 50% even up to 90% of the faeces reaching the rectum each day being swallowed directly from the anus. This accounts for the difference between the fairly rapid "real rate" of faecal excretion of copper, and the very slow "apparent rate" observed when "refected faeces" are passed back into the stomach for further journeys through the tract.

8. In the case of a single dose of 50 mg. of copper as soluble sulphate dried on the food, the "faecal refection cycle" involves high concentration of copper in the tract for several weeks, but despite this there is no evidence of noteworthy absorption, either by increased elimination in the urine or by excessive storage in the liver. The assumption is that the copper is rendered insoluble, probably as the sulphide, by reaction with bacterial decomposition products.

9. The same insolubility factor probably explains the peculiar fact that it is virtually impossible to produce chronic copper poisoning in the rabbit. Although 5 mg. copper as the sulphate injected into the blood stream, or 100 mg. given in dilute solution by stomach tube so as to flush the duodenum with soluble copper, is fatal within a few hours,

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still higher doses such as 200 mg. can be dried on to the food every day for months without producing any effect at all.

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THE VITAMIN D₃ REQUIREMENT OF PULLET CHICKS: THE RELATIVE VALUES OF GENUINE AND A SAMPLE OF CONTROLLED COD-LIVER OIL IN FEEDING POULTRY UP TO THE AGE OF 16 WEEKS

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(With Plates 2 and 3)

AN experiment devised to compare the practical results of feeding chicks on diets containing 1% of the war-time controlled cod-liver oil mixture with the results of feeding the same level of genuine cod-liver oil has given interesting information about the vitamin D₃ requirements of growing pullets for normal calcification.

The two samples of oils were assayed on chicks in accordance with the radiographic technique laid down in the British Standards Institution's specifications No. 911 (Olssen, 1936a, b; Baker & Wright, 1940). The standard used for comparison was therefore the solution of crystalline vitamin D₃ issued by the British Standards Institution. 1 mg. of this solution contains 0.000025 mg. of crystalline vitamin D₃ and represents 1 B.S.I. unit. The vitamin D₃ content of the controlled oil was found to be 56 B.S.I. units per gram and of the pure cod-liver oil 156 B.S.I. units per gram. The limits of error ($P=0.95$) were 88-114%, and the assay therefore was of a high degree of accuracy.

The experiment was divided into two parts; from day-old to 6 weeks and from that age to 16 weeks. During the first 6 weeks the chicks were reared in Moorcoate Battery Brooders in a building from which sunlight was excluded. The relative values of the two oils were judged by their effect on the growth of the chicks and on bone calcification. The hock joints were examined radiographically at 5 weeks old and the tarso-metatarsal distances recorded.

In case the oils proved to be unequally effective on different rations two different chick mashes were used. The two formulae are given below.

In order both to test the margin of safety afforded by each oil and at the same time to conform to common feeding practice, additional grain was fed. Each oil on each formula was tested by (1) an all-mash

1932

Ministry of Agriculture and Fisheries
Bulletin no. 8. Poultry keeping on the general farm, 1930. Intended for use as an all-mash ration

Yellow maize meal	49
Bran	14
Middlings	14½
Meat and bonemeal	7
Dried skim milk	7
Common salt	½
Cod-liver oil	1%

1937

Ministry of Agriculture and Fisheries
Bulletin no. 54. Rearing of chickens, 1937.
 "A useful and economical ration for rearing chicks up to 10 weeks. Well balanced when fed with grain"

Yellow maize meal	20
Wheat bran	30
Wheatings	21
Sussex ground oats	10
Extracted ground earth-nut meal	5
(soya meal unobtainable)	
Dried skim milk	5
Meat meal	5
Common salt	1
Ground limestone	2
Cod-liver oil	1

Note. The formula in the 1932 *Bulletin* requires 1½% cod-liver oil. The 1937 formula advises tested cod-liver oil to be given as 2% of the mash. As controlled cod-liver oil is recommended at 1%, this level was used.

diet, (2) a diet of $\frac{1}{3}$ grain and $\frac{2}{3}$ mash, (3) $\frac{2}{3}$ grain and $\frac{1}{3}$ mash. There were altogether twelve experimental groups as set out below:

1932 formula:

	All mash	$\frac{1}{3}$ grain, $\frac{2}{3}$ mash	$\frac{2}{3}$ grain, $\frac{1}{3}$ mash
Genuine cod-liver oil	"	"	"
Controlled cod-liver oil	"	"	"

1937 formula:

	"	"	"
Genuine cod-liver oil	"	"	"
Controlled cod-liver oil	"	"	"

Chemical analysis of the two mashes gave the following results:

	1932	1937
% ash	5.46	7.60
% calcium	1.32	1.70
% phosphorus	1.10	1.22
Ca/P ratio	1.20	1.39

Dilution of the mashes with grain altered the Ca/P ratio to the following:

	1932	1937
$\frac{2}{3}$ mash, $\frac{1}{3}$ grain	Ca/P = 0.85	Ca/P = 0.98
$\frac{1}{3}$ mash, $\frac{2}{3}$ grain	Ca/P = 0.50	Ca/P = 0.56

PART I

Three hundred and ninety-seven pullet chicks were divided into twelve groups. Each group therefore contained thirty-three chicks (with thirty-four in group I). It was decided that any chicks showing definite rickets should be removed from the experiment. The mortality during the first part of the experiment was:

	1932		1937	
	Genuine cod-liver oil	Controlled cod-liver oil	Genuine cod-liver oil	Controlled cod-liver oil
All mash	3	2	1	5
½ grain	2	8	0	1
⅓ grain	7	33*	9	33*

* During the third week it was evident that an effective feeding level of $\frac{1}{3}$ of 1% of the controlled oil was much below the physiological requirement of the birds on both the 1932 and the 1937 diets. The chicks looked ill and showed deformities of the legs. These birds were therefore photographed as a record (Pl. 2, fig. 1), some representative radiographs of the hock joints of all the groups were made (Pl. 2, fig. 2) and the two groups of rachitic birds were then withdrawn from the experiment.

At this time the comparable chicks on an effective level of $\frac{1}{3}$ of 1% of the genuine cod-liver oil were quite normal in appearance. Radiographic examination of eight chicks drawn at random from each group on the lowest level of genuine cod-liver oil showed, however, that the calcification was defective in two on the 1937 diet and in four on the 1932 diet.

It was desired to examine the calcification by radiography at the end of the first part of the experiment. This was done at 5 weeks instead of 6 weeks old in order to allow sufficient time for collection of the data which were to be used in regrouping the chicks for the second part of the experiment.

Calcification at 5 weeks old (tarsometatarsal distances in millimetres)

	1932 diet		1937 diet	
	Genuine cod-liver oil	Controlled cod-liver oil	Genuine cod-liver oil	Controlled cod-liver oil
All mash:				
Tarsometatarsal distance	0.39	0.51	0.49	0.64
Range	0.16-0.54	0.36-0.76	0.36-0.78	0.33-1.00
Number	31	31	32	26
σ	0.101	0.124	0.185	0.153
ϵ	0.018	0.022	0.033	0.029
½ grain, $\frac{1}{3}$ mash:				
Tarsometatarsal distance	0.37	0.98	0.55	0.96
Range	0.16-0.54	0.34-4.14	0.28-1.50	0.30-3.70
Number	31	25	33	32
σ	0.11	0.878	0.691	0.735
ϵ	0.019	0.176	0.120	0.130
$\frac{1}{3}$ grain, $\frac{1}{3}$ mash:				
Tarsometatarsal distance	0.91	Withdrawn	1.01	Withdrawn
Range	0.34-3.90	"	0.30-3.00	"
Number	26	"	24	"
σ	0.858	"	0.006	"
ϵ	0.168	"	0.185	"

Weights at 5 weeks old in ounces

	1932 diet		1937 diet	
	Genuine cod-liver oil	Controlled cod-liver oil	Genuine cod-liver oil	Controlled cod-liver oil
All mash:				
Weight	8.12	9.52	11.12	11.43
Range	5.25-10.25	7.25-11.50	7.50-13.75	8.50-13.75
Number	31	31	32	28
σ	1.08	1.27	1.27	1.19
ϵ	0.185	0.228	0.225	0.229
$\frac{1}{3}$ grain, $\frac{2}{3}$ mash:				
Weight	7.60	6.81	10.30	8.61
Range	5.25-10.50	4.50-8.50	7.50-13.50	6.25-10.75
Number	31	25	33	32
σ	1.03	1.17	1.27	1.34
ϵ	0.185	0.234	0.221	0.237
$\frac{2}{3}$ grain, $\frac{1}{3}$ mash:				
Weight	6.98	Withdrawn	7.74	Withdrawn
Range	4.25-10.25	"	4.75-10.75	"
Number	26	"	24	"
σ	1.66	"	1.74	"
ϵ	0.326	"	0.363	"

Summary of the findings from the 5-week weights and tarso-metatarsal measurements:

1932 diets

All mash ($\equiv 1\%$ oil).

The weight of the chicks on controlled oil was significantly better than that of those on true cod-liver oil, but there was marked superiority of the genuine oil over the controlled mixture in promoting calcification, used at this 1% level.

$\frac{1}{3}$ grain, $\frac{2}{3}$ mash ($\equiv \frac{2}{3}\%$ oil).

The weight difference in these two groups was not significant, but the controlled oil falls far behind the genuine oil in antirachitic properties.

$\frac{2}{3}$ grain, $\frac{1}{3}$ mash ($\equiv \frac{1}{3}\%$ oil).

The birds on the controlled oil developed severe rickets and were withdrawn from the test at the age of 3 weeks. Those on genuine cod-liver oil showed weights at 5 weeks, as good as the birds on twice this level of controlled oil, and the calcification did not differ significantly in the birds on $\frac{1}{3}$ of 1% genuine cod-liver oil and on twice this amount of controlled oil.

The safety level of genuine cod-liver oil on the 1932 diet.

No significant difference exists between the mean weights and the calcification on 1% and $\frac{2}{3}$ of genuine cod-liver oil. The safety level must be considered to lie somewhere between $\frac{1}{3}$ and $\frac{2}{3}$ of 1%.

The safety level of controlled oil on the 1932 diet.

In appearance and in weight the birds on 1% controlled oil were normal. Their calcification was, however, definitely inferior to that of the birds on cod-liver oil. 1% controlled oil in the complete diet is therefore presumed insufficient to satisfy the full requirements of pullet chicks up to 5 weeks. If grain is fed at the same time as the mash the deficiency is accentuated.

1937 diets

All mash (≡ 1% oil).

There was no significant difference between these two groups in weight, but calcification was significantly better on the genuine cod-liver oil.

$\frac{1}{2}$ grain, $\frac{2}{3}$ mash (≡ $\frac{2}{3}$ % oil).

A significant difference existed in both weight and tarso-metatarsal measurements in favour of the genuine cod-liver oil.

$\frac{2}{3}$ grain, $\frac{1}{3}$ mash (≡ $\frac{1}{3}$ % oil).

The $\frac{1}{3}$ of 1% controlled oil produced severe rickets at 3 weeks and the birds were withdrawn. The birds on $\frac{1}{3}$ of 1% genuine cod-liver oil showed calcification not significantly different from that of birds on $\frac{2}{3}$ of 1% controlled oil.

Safety margins for the two oils on this diet.

For cod-liver oil this lies between $\frac{1}{3}$ and $\frac{2}{3}$ of 1%.

For controlled cod-liver oil it lies somewhere above 1%.

Comparison of the 1932 and 1937 diets.

Growth was better on the 1937 diet than on the 1932 diet, and this difference appears to be significant. Calcification was slightly inferior on the 1937 diet, and this may be because the rather heavier chicks had a higher vitamin D₃ requirement.

The results show that, while on the diets used normal growth can be achieved on 1% of the controlled oil, optimal calcification requires a higher intake.

PART II

The experiment after 6 weeks old

The first part of the experiment having demonstrated the comparative value of the two oils in the early weeks of life, the second half was designed to show the extent to which they would permit birds to reach normal levels of growth and development.

For this purpose the birds were reallocated into twelve groups equal as far as possible in weight and in calcification.

Examination of the weights and tarso-metatarsal distances in these new groups showed that the redistribution was fully satisfactory.

The twelve groups were allocated at random between the same twelve diets and the birds transferred to new quarters in intensive houses, from which sunlight was excluded, at the Agricultural Food Products farm at Bix, Henley.

At 10 weeks old the chick mashes used were changed to rearing mashes, the formulae being taken from the same Ministry of Agriculture publications as before:

	1932		1937
Yellow maize meal	50	Yellow maize meal	20
Bran	16	Bran	31
Middlings	16	Wheatings	20
Meat and bonemeal	4	Sussex ground oats	19
Dried skim milk	4	Ground earth-nut meal	2
Common salt	½	Dried skim milk	2
Cod-liver oil	1	Meat meal	2
Ground limestone	2½	Common salt	1
Steamed boneflour	2	Ground limestone	2
		Cod-liver oil	1

Grain was fed at the same levels as before, but difficulty was experienced in getting the birds to keep to the $\frac{2}{3}$ grain level, and in the last few weeks much of the grain was not eaten.

The 1937 rearing mash was considered less satisfactory in practice than the 1932 formula. The birds finished their allowance rapidly and still appeared hungry. The droppings were looser than on the other diet and the litter needed changing, while that in the pens on the 1932 formula was still fresh. From the eleventh week all the groups were fed *ad lib.* but still maintaining the proportions of mash and grain.

The birds were weighed at 9, 11, 13 and 16 weeks old. At the same time each bird was graded as class I, II, III or IV, on grounds other than weight—i.e. on appearance, body development, feathering, eye condition, etc. Examples are given in Pl. 3.

The results of the weighings were as follows:

Mean weights from the 6th to the 16th week

Week	1932 diet		1937 diet	
	Genuine cod-liver oil	Controlled cod-liver oil	Genuine cod-liver oil	Controlled cod-liver oil
All mash				
6	12.11	12.11	*12.11	12.11
9	24.04	20.58	21.82	19.95
11	32.52	31.91	31.37	29.15
13	42.20	38.87	39.41	38.80
16	50.60	49.50	43.70	47.50
Gain 6-16 week	38.49	37.39	31.49	35.39
$\frac{1}{2}$ grain				
6	12.11	12.11	*12.11	12.11
9	24.50	22.55	22.52	22.82
11	32.21	32.36	31.41	31.14
13	40.58	39.09	39.86	39.77
16	47.10	47.70	47.30	50.00
Gain 6-16 week	34.99	35.59	35.19	37.89
$\frac{2}{3}$ grain				
6	12.11	12.11	12.11	12.11
9	22.13	21.53	21.79	22.04
11	28.77	29.50	29.32	29.14
13	34.33	37.24	33.19	34.86
16	42.30	43.30	44.10	42.40
Gain 6-16 week	30.19	31.19	31.99	30.29

The mean gains in weight made by the twelve groups were therefore very similar over the 10 weeks. The progress of two starred groups suffered, however, during the last 10 days of the test by appearance of acute coccidiosis. The all-mash group was the more severely affected. This undoubtedly prevented part of the progress these two groups should have shown in the last weighing. No deaths were recorded, but group 1937 (1% genuine cod-liver oil) definitely lost condition. Coccidial forms were identified in their blood-stained droppings and all measures possible were taken to prevent spread of the disease.

Grading

The twelve groups were compared according to the quality of the birds by awarding 4 marks for a class I bird, 3 for class II, 2 for class III and 1 for class IV. The marks were added, multiplied by 100 and divided by the number of birds per group. The results were:

Week	1932 diet		1937 diet	
	Genuine cod-liver oil	Controlled cod-liver oil	Genuine cod-liver oil	Controlled cod-liver oil
All mash				
9	300	308	300	265
11	308	283	319	290
13	332	279	341	250
16	313	292	332	330
Totals	1253	1162	1292	1135
$\frac{1}{2}$ grain				
9	313	291	281	277
11	283	273	268	282
13	254	218	345	323
16	233	214	324	333
Totals	1083	996	1218	1215
$\frac{2}{3}$ grain				
9	239	289	238	268
11	255	282	232	218
13	233	259	196	191
16	180	241	148	171
Totals	907	1071	814	848
Total for all three levels:				
	3243	3229	3324	3198

Feathering

The best feathering was seen in the birds on 1% of genuine cod-liver oil on both diets, but the feathering on 1% of both oils was satisfactory in all cases. It tended to be dry and loose in some birds when $\frac{1}{2}$ of grain and $\frac{2}{3}$ mash was fed. On $\frac{2}{3}$ grain and $\frac{1}{3}$ mash on both diets certain birds showed large unfeathered patches and were subject to cannibalistic attack by their fellows. The groups in which this was noticeable were those on controlled cod-liver oil mixture and on the $\frac{1}{2}$ of 1% level of genuine cod-liver oil (1937 diet).

Calcification

No leg weakness was seen in any group, but it was desired to determine to what extent normal calcification had been produced by the two oils. The hock joints of eight birds from each group were examined radiographically, making a total of forty-eight on genuine and forty-eight on controlled oil, chosen at random.

Five only of these birds showed really bad calcification. These all occurred on the controlled cod-liver oil mixture, two on $\frac{1}{2}$ grain and $\frac{2}{3}$ mash and three on $\frac{2}{3}$ grain and $\frac{1}{3}$ mash. The loss of detail in bone structure is seen by comparison of the radiographs in Pl. 2, fig. 3.

The subsequent records of birds which showed defective calcification at 5 weeks old were individually examined.

The 16 week weights and grades of the birds which showed defective calcification at 5 weeks old

1932 diets						1937 diets					
Genuine cod-liver oil			Controlled cod-liver oil			Genuine cod-liver oil			Controlled cod-liver oil		
Bird no.	oz.	Class	Bird no.	oz.	Class	Bird no.	oz.	Class	Bird no.	oz.	Class
All mash											
337	43	II	350	41	IV	358	31	III	501	36	II
504	38	III	571	45	III				598	38	IV
$\frac{1}{4}$ grain											
456	36	IV	342	33	IV	496	47	I	512	45	II
			552	55	II	455	41	I	597	40	III
$\frac{2}{3}$ grain											
359	46	IV	497	35	IV	339	35	V	513	36	IV
490	41	II	423	50	III	501	40	III	Very bad feathering 450	47	III
									580	38	IV
									590	38	III

It was thus exceptional for a bird rachitic at 5 weeks old to become a first quality bird at 16 weeks. The only two which achieved this did so on $\frac{1}{4}$ grain and $\frac{2}{3}$ mash with genuine cod-liver oil.

SUMMARY AND CONCLUSIONS

Calcification on 1% of controlled cod-liver oil was less satisfactory than on 1% of genuine cod-liver oil as judged by radiography at 5 weeks old. Growth rates were not significantly different.

$\frac{2}{3}$ of 1% of the genuine cod-liver oil promoted calcification as satisfactorily as (and significantly better on one diet than) 1% of controlled oil. The mean tarso-metatarsal distances on $\frac{2}{3}$ of 1% of controlled oil were too wide for the calcification to be regarded as normal. It has thus been shown that satisfactory growth is no criteria of adequate calcification and bone structure.

From the age of 6-16 weeks, $\frac{2}{3}$ of 1% of controlled cod-liver oil mixture was not adequate, as demonstrated by radiographic examination, for optimal calcification; but using the criteria of naked eye examination and weight, no significant difference would have been found between genuine cod-liver oil and the controlled mixture. These birds showing subnormal calcification could not be expected to stand up well to the strain of laying, which puts heavy demands upon the calcium metabolism.

Since the vitamin D₃ values of the oils were known, it may be stated that, on the two diets used, normal calcification was produced in 5-week-old chicks by 104 B.S.I. units (0.0039 mg. crystalline vitamin D₃) per 100 g. total diet—supplied in $\frac{2}{3}$ of 1% of the genuine cod-liver oil—but not by 56 B.S.I. units (0.0014 mg. crystalline vitamin D₃) per 100 g. total diet, supplied in 1% of the controlled cod-liver oil.

Severe rickets arose on 19 B.S.I. units (0.00047 mg. crystalline vitamin) per 100 g. total diet ($\frac{1}{3}$ of 1% of the controlled oil). Normal calcification at 15 weeks old is not produced in all birds by 38 B.S.I. units (0.00095 mg. crystalline D₃) per 100 g. mash, supplied in $\frac{2}{3}$ of 1% controlled oil.

It would appear, therefore, that the vitamin D₃ requirement for optimal calcification cannot be far short of 100 B.S.I. units per 100 g. total diet. This applies to the actual diets used. It is possible that in practice, using less satisfactory mashes, a margin over this might be needed.

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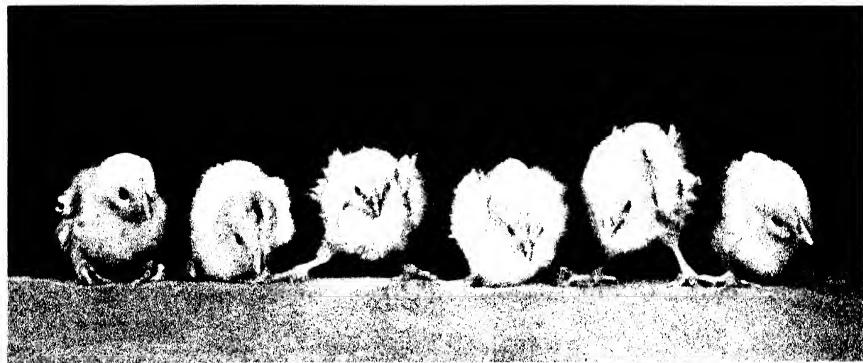
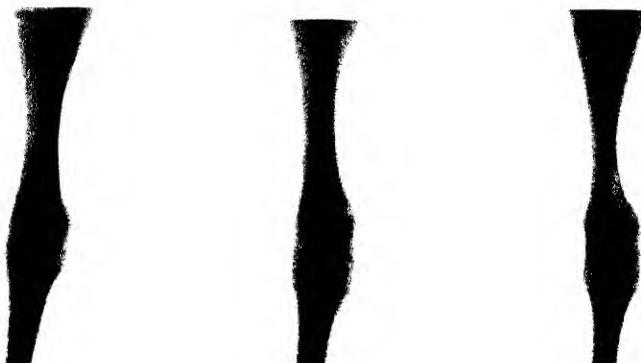


Fig. 1. Rachitic chicks at 3 weeks old.



Normal hock joint.

Hock joints from rachitic birds.
Fig. 2.

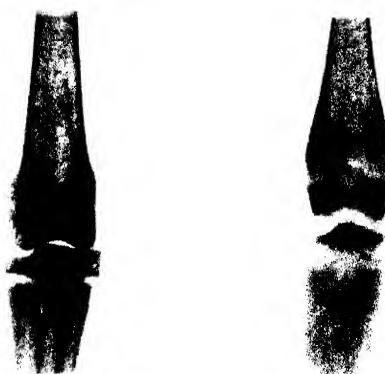
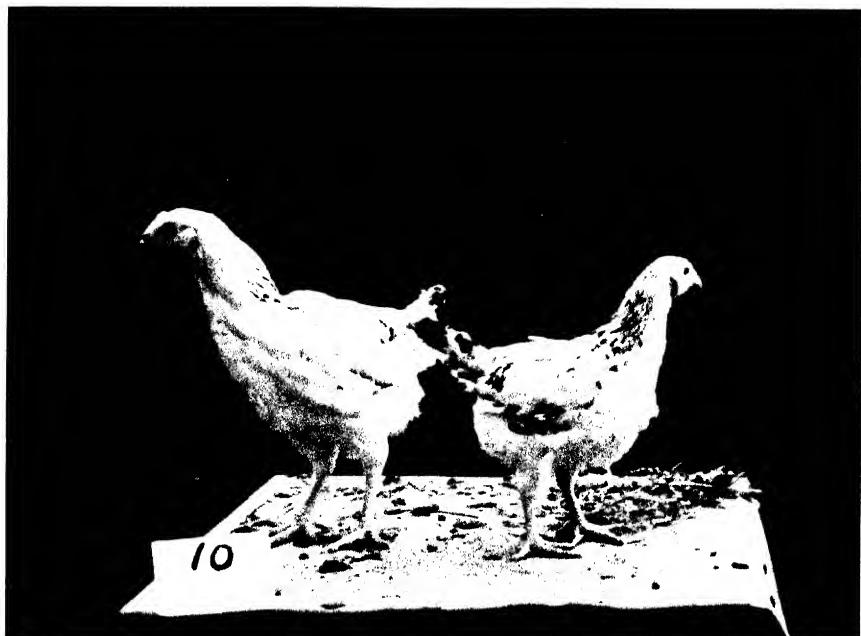
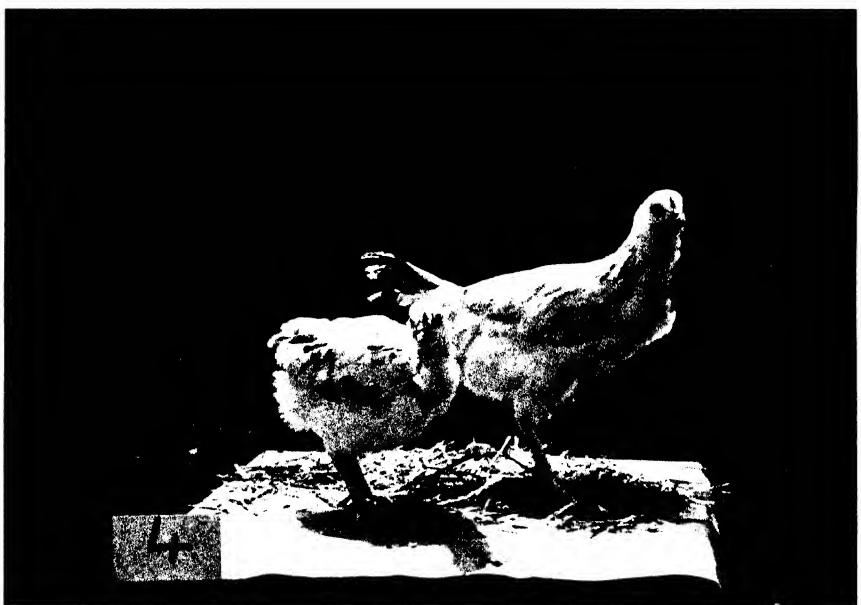


Fig. 3. Normal and defective calcification at 15 weeks old.



Classes I and III.



Classes I and IV.

ULTRA-MECHANICAL ANALYSIS OF SOILS

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THE usual methods of mechanical analysis of soils, including the hydrometer or the pipette methods, have often been used for particles down to 0.001 mm. diameter. This size is larger than the upper limit of colloidal particles, which is 0.0005 mm. It is, however, possible to push the mechanical analysis to such extreme limits by gravity sedimentation by extending the time of settlement to several weeks. The use of the centrifuge in accelerating sedimentation would also suggest itself. For the ordinary mechanical analysis it was used to some extent before the general theory of sedimentation was fully developed (Briggs *et al.* 1904).

The modification of the pipette method to include a sedimentation tube which may be centrifuged is not possible. The reason is not far to seek; the pipette method is based on the assumption that all particles of the same size settle with the same velocity at all depths. While this is true for gravity settlement, it is not true for sedimentation in a centrifuge tube, for the settling velocity of the particles is proportional to the distance from the axis of rotation. One way of overcoming this difficulty would be to make the length of the sedimenting column small as compared with its distance from the axis of rotation. This would mean that if the sedimenting tube is 10 cm. long the axis of rotation should be at least 100 cm. from the bottom of the tube. Although it is not impossible to construct a centrifugal machine with a span of 2 m., the instrument could hardly be accommodated on a laboratory bench. As such an instrument might have to run for several hours, the vibrations and noises would necessitate its housing in a special room.

Marshall (1930) has recently tried to overcome this limitation by putting a thin layer of the soil suspension from which all the coarser particles had been removed on top of a thick layer of a denser liquid, such as sugar or urea solution, and centrifuging this. Under these conditions, according to Marshall, the soil particles reaching the bottom of the tube between any two given times all lie within the two limits of diameter calculated from these times.

The simplicity of gravity sedimentation and the application of the pipette method to the ultra-mechanical analysis of soils though pointed

out by Robinson (1922) has so far escaped attention on account of the long time of settlement. If this could be reduced the technique would be ideal. This is only possible by reducing the depth at which pipetting is done. Hitherto, it has not been found practicable to have it less than 2.5 cm., but there seems no reason why it could not be reduced to 0.5 cm., or even 0.1 cm. The actual pipetting could be so refined that only the top millimetre or so of the surface is removed. This would reduce the time factor considerably. For instance, we could catch particles of 40 μ diameter by pipetting at 1 mm. after 1 week's settling, which would normally have taken 50 weeks if the pipetting was done at the customary 5 cm. depth. The equivalence of time/depth ratio has been shown for clay up to 2.5 cm. (Puri & Amin, 1928), but smaller depths have not been attempted.

The present paper describes a micro-pipette which has been successfully used for pipetting at extremely small depths and with the help of which the equivalence of time/depth ratio has been established down to 1 mm. depth.

DESCRIPTION OF THE APPARATUS

The micro-pipette consists of a 10 c.c. pipette with a hypodermic needle attached to the tip. The pipette is provided with a two-way tap at the suction end, so that when it is closed the pipette holds exactly 10 c.c. of the suspension, the excess flowing out via the other end of the tap. It is mounted on a rack and pinion stand capable of controlled movement to the fraction of a millimetre. The pipette can be moved by two screws, one of which causes it to move against a graduated scale with vernier attachment. The pipette is first moved down with one screw until the tip of the hypodermic needle just touches the surface of the suspension. This point is extremely sharp and can be reproduced to the fraction of a millimetre. From this point the second screw is rotated and with the help of the vernier scale the tip of the needle is let down to any desired depth accurately. The suction is applied with the help of a mercury reservoir attached to a bulb which is alternately filled and emptied by raising or lowering the reservoir. The rate at which the pipette is filled is controlled by the tap leading to the reservoir. When the pipetting is complete the mercury pressure is relieved by raising the reservoir and the pipette emptied by detaching the hypodermic needle and thus widening the opening. The pipette is first calibrated to deliver exactly 10 c.c. which is quite enough for evaporating to dryness in a crucible

and weighing the residue. A hypodermic needle of 1 c.c. capacity can be joined by the help of a stout rubber tube or bung straight on to a 10 c.c. pipette having its tip filed off. There is sometimes air leak on the ground-on joint between the hypodermic needle and the tip of the syringe, but it gives no trouble if it is kept well greased. When it shows signs of wear, it should be replaced by a new syringe.

As we are concerned only with the top portion of the liquid at no great depth the use of tall sedimenting cylinders is obviously not appropriate. Squat bottles, which are wide but not too tall, are used for holding the suspension. If the diameter of the bottle is 16 cm., the removal of 10 c.c. of the suspension by pipetting makes a difference of only 0.5 mm. in the height of the liquid.

EQUIVALENCE OF TIME/DEPTH RATIO

Particles of 0.001 mm. diameter were determined in nine soils by pipetting at 1, 3, 4, 5 mm., 1.0, 2.5, and 5.0 cm. depths and in eighteen soils by pipetting at 1 mm. and 5.0 cm. depths, by allowing the suspension to settle for various lengths of time appropriate for each depth.

The results given in Table 1 leave no doubt that clay (0.001 mm.) can be determined by pipetting with this technique even at the depth of 1 mm. from the surface. The time of sampling is thus reduced to about 15 min. only instead of several hours. There is very good agreement between the various replicates. Actually the same suspension was pipetted at various times at increasing depths which shows that there is no disturbance produced in the suspension by micro-pipetting. It might be mentioned that the tip of the hypodermic needle has a nib-shaped point, which is rather an advantage, as the suction creates horizontal stream lines and the disturbance is the least. It must be remembered,

Table 1 A. *Showing equivalence of time/depth ratio*

Soil no.	Depths of pipetting							
	1 mm.	2 mm.	3 mm.	4 mm.	5 mm.	1 cm.	2.5 cm.	5 cm.
P.C. 13	58.4	57.2	57.9	57.4	59.1	58.0	59.6	58.6
P.C. 107	33.4	30.0	31.1	30.6	30.3	30.1	30.2	30.2
P.C. 109	24.4	24.9	24.7	24.0	22.5	22.8	23.0	22.6
P.C. 110	12.3	10.8	12.6	11.4	12.2	12.5	13.0	12.2
P.C. 111	10.0	8.9	8.0	8.7	8.9	9.0	7.9	8.8
P.C. 116	26.5	26.5	24.3	25.6	23.0	24.0	24.4	24.8
P.C. 118	27.8	26.9	25.7	27.4	26.1	26.4	26.0	25.8
P.C. 123	82.1	78.9	81.0	80.8	80.7	81.3	81.0	81.0
P.C. 142	59.9	58.3	56.8	57.4	58.0	58.3	59.2	58.8

Table 1 B

Soil no.	Depths of pipetting	
	1 mm.	5.0 cm.
	% 0.001 mm.	
P.C. 126	30.6	30.2
P.C. 127	27.0	25.6
P.C. 128	34.8	35.2
P.C. 129	29.8	30.9
P.C. 130	34.0	32.2
P.C. 131	35.2	32.6
P.C. 132	27.5	25.9
P.C. 133	8.2	8.4
P.C. 134	8.8	8.8
P.C. 135	9.1	8.5
P.C. 136	13.6	14.0
P.C. 137	14.8	14.8
P.C. 139	12.0	12.8
P.C. 140	13.0	12.6
P.C. 241	14.2	14.0
P.C. 242	18.0	15.8
P.C. 243	12.6	11.1
P.C. 244	40.2	37.0

however, that the actual opening is above the tip and allowance should be made for this distance in letting down the tip to the proper depth at the time of sampling.

ULTRA-MECHANICAL ANALYSIS OF SOILS

The time taken by particles of various sizes below 0.001 mm. to settle to a depth of 1 cm. from the surface at various temperatures can be calculated from Stokes's law,

$$V = \frac{2}{9} \frac{gr^2 (d' - d)}{\eta},$$

where V = rate of fall, g = gravitational acceleration, r = equivalent radius of the particles, d' = density of particles ($= 2.68$), d = density of water ($= 1.0$), and η = viscosity of water.

Remembering that the equivalence of time/depth ratio holds down to even 1 mm. depth, the time of settlement for any depth can be easily calculated.

When dealing with ultra-clay particles, it is more convenient to plot the negative indices of the settling velocities in cm./sec. or diameters in cm. against the summation percentages. The latter may be conveniently represented by the symbol pD ; it was discussed in a previous paper (Puri, 1939).

The ultra-mechanical analysis of four soils was determined by (1) the

micro-pipette method, (2) the centrifugal method using Marshall's technique, and (3) the ordinary pipette technique in which the longest time of settlement was three months. The results are given in Table 2. In spite of the fact that Marshall's method makes use of a somewhat different principle and the ordinary pipette technique involves a long time of settlement and thus is subject to unavoidable errors due to temperature changes, the results (Table 2) are sufficiently close to establish the claim of the micro-pipette method for being adopted for routine analysis.

Table 2. *Ultra-mechanical analysis of soils by (1) micro-pipette method, (2) Marshall's technique, and (3) ordinary pipette method*

Soil no.	Size of particles (in pD)											
	4.2			4.6			5.0			5.2		
	Method no.			Method no.			Method no.			Method no.		
	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)
P.C. 13	54.5	54.4	52.5	50.2	49.0	47.0	45.8	44.2	45.6	36.6	37.2	—
P.C. 116	22.4	19.6	18.0	18.1	13.8	16.2	15.0	10.2	12.7	7.6	7.2	—
P.C. 123	74.6	69.8	82.0	52.9	51.6	61.2	30.2	34.1	40.5	19.2	22.0	—
P.C. 142	56.0	51.6	51.9	47.8	42.9	44.6	34.3	33.6	42.1	22.9	21.2	—

Its superiority over Marshall's technique lies in the simplicity and the rapidity with which the analysis can be done. For instance, using Marshall's technique, it took the authors no less than 3 days to determine the percentage of the particles of only four sizes corresponding to pD 5.2, 5.0, 4.6 and 4.2 in a single soil. The machine was worked at 2000 rev./min., and 20% sugar solution was used as the lower liquid. Besides this, it was found very inconvenient to have repeated precipitations with hydrochloric acid and decantations in order to free the sediment from sugar. Marshall's method, thus, at best is unnecessarily elaborate, costly and time consuming.

The micro-pipette method, on the other hand, is simple, straightforward and theoretically as sound as the well-known pipette method. A dozen or more samples can be started at the same time and put aside for settling and pipetted one after the other at appropriate depth/time intervals. The authors take the diameters corresponding to pD 4.0, 4.2, 4.6, 5.0, 5.2, and 5.4. The lowest diameter is thus 0.0000398 mm., i.e. a little less than 40 m μ . This size would take little more than 7 days to settle through 1 mm. depth at 25° C. The authors find it convenient to determine particles corresponding to pD 4.6, 4.2 and 4.0 by allowing the suspension (at 25° C.) to settle for 5 hr. and 25 min. and pipetting off at 1.25, 8.0 mm. and 2 cm. depths. The suspension is again shaken and left

for 7 days and 3 hr. and then pipetted at 1, 2.5 and 6.3 mm. in order to determine particles corresponding to pD 5.4, 5.2 and 5.0 respectively.

A word about the method of dispersion of the soil for ultra-mechanical analysis. It has been shown elsewhere (Puri *et al.* 1938) that for the disintegration of ultra-clay treatment with acid followed by shaking with NaOH to pH 10.8 is absolutely essential. The simplest procedure, therefore, is the treatment of the soil with 0.05 N HCl till free from exchangeable calcium and followed by shaking with sufficient NaOH solution to bring the pH value of the suspension to 10.8. The significance of this pH value has been discussed in another paper (Puri & Lal, 1939). For calcareous soils a stronger solution of HCl can be used as a preliminary treatment for decomposing the major portion of carbonates.

EFFECT OF OXIDIZING AGENTS ON THE ULTRA-MECHANICAL ANALYSIS

Preliminary treatment of the soil with oxidizing agents like H_2O_2 or $KMnO_4$ is frequently resorted to for the destruction of the organic matter. The oxidation with H_2O_2 is admittedly mild, but oxidation with permanganate might easily result in a destruction of a part of the inorganic soil colloids. This adverse effect would naturally be confined to particles of ultra-clay. It was, therefore, of interest to study the effect of this treatment on the ultra-mechanical analysis. Nine soils were used for this study. These soils were not rich in humus as the object was to find out what effect the treatment had on the inorganic colloids. The soils were treated with alkaline permanganate in accordance with the directions given in a previous paper (Puri & Sarup, 1937). Ultra-mechanical analysis of the soils before and after treatment were compared. The results given in Table 3 show that oxidation with alkaline $KMnO_4$ has not produced any striking difference in the ultra-clay.

Table 3. *Effect of oxidizing agents on the ultra-mechanical analysis*

Soil no.	Percentage of ultra-clay (in pD)											
	4.0		4.2		4.6		5.0		5.2		5.4	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
P.C. 13	58.6	53.4	54.5	50.8	50.2	46.8	45.8	37.8	36.6	31.8	26.4	25.5
P.C. 116	24.8	23.3	22.4	20.4	18.1	16.9	12.7	11.0	7.6	6.6	6.0	5.6
P.C. 130	32.2	31.0	30.8	27.2	27.8	23.2	20.5	16.5	14.0	10.0	12.9	7.2
P.C. 183	8.4	10.2	7.0	9.2	6.1	8.3	4.1	3.5	3.5	3.1	0.8	1.2
P.C. 242	15.8	15.2	13.5	13.2	11.2	11.9	7.0	5.4	5.6	4.0	4.5	3.2
P.C. 243	11.1	9.5	10.0	8.3	8.0	6.3	3.6	4.5	2.6	2.0	2.0	2.0
P.C. 244	37.0	36.5	29.0	30.8	20.1	21.5	12.4	12.6	9.0	9.6	5.2	7.6
P.C. 245	30.1	34.8	28.6	29.0	20.5	20.9	12.6	13.5	10.2	9.6	9.8	7.2
P.C. 250	8.2	10.4	8.0	9.0	6.0	7.2	4.5	3.8	4.0	3.1	3.6	2.8

* (1) Untreated. (2) Treated with permanganate.

SUMMARY

A micro-pipette for the ultra-mechanical analysis of soils is described. The sources of error in the usual centrifugal methods are discussed and the new method is shown to be simple, accurate and capable of being adopted as a routine method even in the most moderately equipped laboratories. The treatment of the soil with alkaline permanganate to destroy humus does not affect the inorganic soil colloids.

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THE CHEMICAL
EVALUATION OF PYRETHRUM FLOWERS
(*CHRYSANTHEMUM CINERARIIFOLIUM*)

THE EXTRACTION OF THE FLOWERS FOR ANALYSIS AND
THE PREPARATION OF COLOURLESS CONCENTRATES OF
THE PYRETHRINS

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(With One Text-figure)

INTRODUCTION

In the determination of the pyrethrins, the insecticidal principles of pyrethrum flowers, it is customary to extract the ground flowers with low-boiling petroleum ether. This solvent is used because of its selective action, extracting the active principles with a minimum of extraneous matter. Ripert (1934), however, has suggested that owing to the protective action of oxyacids, formed particularly on storage and insoluble in petroleum ether, the pyrethrins may not be completely removed by this solvent, and has found (1936) a chloroform extract of the flowers made after extraction with petroleum ether to be highly toxic to houseflies. Gnadinger (1936) points out the possibility of the extraction by ether of changed pyrethrins, reacting in the analytical methods as true pyrethrins, but of lower toxicities. The position has been discussed, from the chemical point of view, in a preliminary way by Martin (1938). The present communication gives the results of biological trials carried out on ether extracts of the flowers made after preliminary extraction with petroleum ether.

We have shown (Martin & Potter, 1937) that if the powdered flowers are intimately mixed with decolorizing charcoal, and then extracted in a Soxhlet apparatus with petroleum ether, a colourless extract results. Further information as to the nature and pyrethrin contents of such extracts is now given. The work was undertaken because of the need, for a separate investigation, of a concentrate of the pyrethrins, and it was considered that a colourless extract, made by the extraction of flowers admixed with charcoal, would provide a suitable starting material for this work.

EXPERIMENTAL

The extraction of the flowers for analysis

Biological tests. The flowers consisted of *Chrysanthemum cinerarium-folium* showing on analysis by the Seil method, without the removal of free acids, 0.45% pyrethrin I, and 0.59% of pyrethrin II, expressed on a dry-matter basis. An aliquot of 10.0 g. of the air-dried ground flowers was extracted by percolation with low-boiling petroleum ether for 2½ hr. The percolation was then continued with fresh solvent for a further 3 hr., the second extract being coloured pale yellow. The extracts were combined, the solvent removed by distillation from a water bath, and finally under reduced pressure.

The powdered material was dried at 30–35° C. for 1 hr., and then percolated with ether for 3 hr. The solvent was removed from the greenish yellow extract as before. The resins were taken up, with warming, with four successive portions of absolute ethyl alcohol, each extract being cooled and strained through cotton-wool to remove fatty material. The filtrates were made up to 80 ml. in the case of the petroleum-ether extract and 20 ml. in the case of the ether extract. The alcoholic solutions were diluted with 0.5% saponin and the alcohol content of the spray solutions adjusted to 20 or 25%.

Adults of *Tribolium castaneum*, reared on whole-meal flour in a constant-temperature room at 27° C. and 70% R.H., were used as test subjects. The insects were sprayed on flannelette in the machine described by Tattersfield (1934), using for each concentration five replicates, each of 15–20 insects. They were returned in small tubes covered with muslin to the constant-temperature room and examined 24 hr. later. Insects that were badly affected, moribund or dead were taken together in assessing the mortality. The results are given in Table 1.

There is obviously no residual toxic action to this insect after percolation of the flowers with petroleum ether for 5½ hr.

Further trials were carried out using *Aphis rumicis* as test subject. The flowers used were grown on our experimental plot at Woburn, Beds, and had been standing in the ground condition in a tin in a cool place for nearly 12 months. 15 g. were percolated for 3 hr. with low-boiling petroleum ether, the free acids were removed by washing the extract with 0.1 N NaOH, and the Seil method carried out, using an excess of 1 ml. N H_2SO_4 for acidification before the distillation of the mono-carboxylic acid. The sample showed 0.59% of pyrethrin I and 0.61% of pyrethrin II. The petroleum-ether-extracted material was air-dried at

Table 1. Toxicities to *Tribolium castaneum* of petroleum ether and of ether after petroleum-ether extracts of pyrethrum flowers

% of flowers	% alcohol content of spray solution	% mortality allowing for control	% S.E.*
Petroleum-ether extraction			
1.41	20	68.9	7.0
1.12	20	62.3	13.9
0.84	20	25.0	2.4
0.56	20	22.7	7.3
0.28	20	9.3	4.3
Ether after petroleum-ether extraction			
12.5	25	0	—
10.0	20	0	—
7.5	20	4.0	—
Control, alcohol-saponin			
—	25	5.7	—
—	20	1.2	—

* Calculated on percentage mortalities before allowing for control.

35° C. for $2\frac{1}{2}$ hr., and percolated for 3 hr. with ether. Free acids were removed and the ether extract subjected to the Seil method as before; 0.04 % of apparent pyrethrin I and 0.12 % of apparent pyrethrin II resulted. A duplicate aliquot of 15 g. was extracted in the same manner with petroleum ether and then with ether, and the solvents removed, finally under reduced pressure. The resins were weighed (petroleum-ether extract 4.8 %, subsequent ether extract 3.1 %) and taken up with successive portions of warm absolute alcohol, with cooling and filtration through cotton-wool. The filtrate from the petroleum-ether extraction was made up to 100 ml. and that from the ether extraction to 50 ml. The resins were tested in 0.5 % saponin containing 5 % of alcohol. For each concentration five replicates each of ten insects were used. The percentages of badly affected, moribund and dead insects recorded 44 hr. after spraying were taken in assessing the mortalities. The results are given in Table 2.

The regression lines relating probits (Bliss, 1935) to the log concentrations in the spray fluids of petroleum ether and ether-extracted resins, pyrethrin I and total pyrethrins are given in Fig. 1 A, B and C respectively.

Tribolium castaneum is much less susceptible to pyrethrum than is *Aphis rumicis*. The concentration of pyrethrin I required to kill 50 % of *Tribolium castaneum* was of the order of 0.0048 %, while the median lethal concentration for *Aphis rumicis* was of the order of 0.0003 %.

The value of 0.04 % of apparent pyrethrin I extracted by ether after

Table 2. *Toxicities to Aphis rumicis of petroleum ether and ether after petroleum-ether extracts of pyrethrum flowers*

% of flowers	% of resin	% pyrethrin I for control	% mortality allowing for control	% s.e.*
Petroleum-ether extraction				
0.23	0.0108	0.0012	100	—
0.15	0.0072	0.0009	100	—
0.075	0.0036	0.0004	75.8	3.7
0.038	0.0018	0.0002	35.1	3.3
Ether after petroleum-ether extraction				
1.50	0.0458	0.0006	70.3	5.7
1.13	0.0344	0.0005	62.6	4.1
0.75	0.0229	0.0003	26.3	14.5
0.45	0.0138	0.0002	32.9	12.2
0.15	0.0046	0.0001	14.7	6.3
Control, alcohol-saponin				
—	—	—	10.0	—

* Calculated on percentage mortalities before allowing for control.

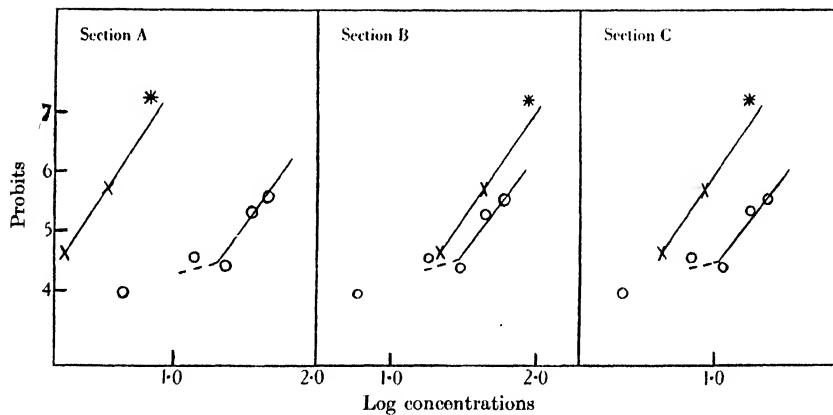


Fig. 1. Probits plotted against the log concentrations of the resins extracted by petroleum ether and subsequent ether extraction (Section A), of pyrethrin I (Section B) and of total pyrethrins (Section C).

× Petroleum-ether extraction. ○ Subsequent ether extraction.
* Calculated value for 100 % mortality.

petroleum ether is only just outside the experimental error of the Seil method. A definite toxic effect to *Aphis rumicis* was obtained when the ether-extracted resin was tested at high concentrations, but judging from the concentrations required for 50 % mortality of the insects (Fig. 1), the original flowers were of the order of 18 times more toxic than the same flowers after petroleum-ether extraction. The ether-extracted resin was slightly less toxic, by comparison with the petroleum-

ether-extracted resin, than its apparent pyrethrin I content would have indicated, and was definitely overvalued by its apparent total pyrethrin content. It is probable that the value of 0.12 % is an overestimate of the amount of true pyrethrin II occurring in the ether-extracted resin. Extraction by percolation with petroleum ether for 3 hr. has thus accounted for something like 95 % of the biological activity of the flowers tested. In view of its selective action, this solvent should therefore be retained for the extraction of the flowers for analysis, a minimum period of 8 hr. being employed.

The preparation of colourless concentrates of the pyrethrins

Comparison of petroleum ether and chloroform extracts of the flowers, with and without admixture with charcoal. The flowers used were taken from an experimental bed of pyrethrum at Harpenden, and were characterized by a high content of pyrethrin I in comparison with that of II. A portion of 37.5 g. was exhaustively extracted, in subdued light, in a Soxhlet apparatus with low-boiling petroleum ether. Further portions of 37.5 g. were intimately mixed with 2.5 and 5.0 g. of decolorizing charcoal, freshly received from the manufacturers, and similarly extracted with petroleum ether. The extracts were made up to 250 ml. and 100 ml. aliquots used for the tests.

Free acids were removed by the addition of water to the petroleum-ether solution in a separating funnel and titration with 0.02 N alkali to phenolphthalein. There was a distinct tendency for the formation of an emulsion in the case of the flowers extracted directly with petroleum ether, but not where the flowers had been admixed with charcoal. The petroleum-ether solutions were washed, dried, the solvent removed and the resins recovered and weighed.

Saponification was carried out with 0.5 N methyl alcoholic potash, the alcohol was removed by warming under reduced pressure, and the pyrethrins determined by the method of Seil (1934) using 1-2 ml. of normal acid for the acidification of the filtrate from the barium precipitation. The results are given in Table 3.

Further tests were carried out, using the same flowers but with chloroform as solvent. In this case, it was found necessary to increase the (fresh) charcoal content of the mixture to at least 45 % in order to obtain an almost colourless extract. The results are given in Table 4.

The comparison of the pyrethrin contents of the flowers given by direct petroleum-ether and chloroform extraction is of interest. Chloroform extraction has resulted in only a slightly higher value for pyrethrin I,

but in an appreciably higher figure for pyrethrin II. This effect has been noted before (Martin, 1938) when the pyrethrin contents of flowers given by petroleum ether and ether extraction were compared. It is unlikely that petroleum ether exerts a preferential extraction of pyrethrin I from the flowers. In view of the additional fatty material extracted by ether or chloroform, it is probable that the resulting values for pyrethrin II are overestimates of this constituent, a proportion of fatty acid, non-volatile in steam and soluble in ether being determined with the di-carboxylic acid.

Table 3. *Comparison of petroleum-ether extracts of the flowers, with and without charcoal*

Colour of extract	No charcoal	Charcoal, % of mixture	
						6.3	11.8
Resin % of flowers					Deeply coloured	Very pale yellow	Colourless
ml. of 0.02 N alkali to neutralize free acids						2.9	2.0
Pyrethrin I % of flowers						3	2
Pyrethrin II % of flowers						0.87	0.74
Total pyrethrins % of resin						0.31	0.17
					32	39	45

Table 4. *Comparison of chloroform extracts of the flowers, with and without charcoal*

Colour of extract	No charcoal	Charcoal, % of mixture	
						45 % of mixture	Pale yellow
ml. of 0.02 N alkali to neutralize free acids					Deeply coloured	25	8
Resin % of flowers						8.27	4.75
Pyrethrin I % of flowers						0.88	0.85
Pyrethrin II % of flowers						0.52	0.39
Total pyrethrins % of resin						17	26

It is clear from Table 3 that a colourless extract obtained by the use of charcoal containing 45 % of pyrethrins, and a reduced content of substances with emulsifying properties should be readily amenable to further purification in the preparation of pyrethrin concentrates. Further work on such extracts was therefore carried out, based upon the methods used by LaForge & Haller (1935).

Preparation of pyrethrin concentrates. In a preliminary test, 50 g. of flowers mixed with charcoal was extracted with petroleum ether. In this case, charcoal which had been standing in the laboratory for a considerable time was used, and it was found necessary to incorporate approximately 35 % of charcoal in the mixture before a colourless extract was obtained. Free acids were removed and the colourless resin, dissolved

in acetic acid containing 10 % of water, was cooled to 0° C. The fatty material separating was removed, and an oil recovered by dilution of the filtrate with water and extraction with petroleum ether.

The total pyrethrin content of the colourless oil, determined by the Seil method, was 78 %, made up of 59 % of I, and 19 % of II.

A second experiment was carried out, in which 700 g. of flowers were mixed with 420 g. of charcoal (old) and extracted with petroleum ether. A pale yellow extract resulted. On concentration and refrigeration for some days, white crystals separated. These were filtered off and the free acids removed from the filtrate with dilute alkali. The resin (14 g.) was recovered and dissolved, with warming, in glacial acetic acid. On cooling, further crystalline material separated. This was filtered off and washed with acetic acid; 10 % of water was then added to the filtrate and the solution cooled to 0° C. Fatty material separating was filtered off. The acid solution was then extracted with two volumes of petroleum ether, the petroleum-ether solution washed four times with acetic acid containing 10 % of water, and then repeatedly with water. The solution was dried over sodium sulphate and the solvent removed, to yield 4.2 g. of a pale yellow oil.

This showed by the Seil method a total pyrethrin content of 78 %, made up of 65 % of I and 13 % of II.

The oil (2 g.) was dissolved in petroleum ether and slowly run through a column of charcoal ($3 \times 1\frac{1}{4}$ in.) previously wetted with the solvent. The charcoal was washed repeatedly by percolation with petroleum ether. On removal of the solvent from the percolate, 0.1 g. in all of a colourless aromatic oil resulted. The charcoal was then percolated for two periods of 6 hr. each with ether. The first extraction yielded 1.33 g. and the second 0.20 g. of a colourless oil. The charcoal, after mixing, was percolated for $7\frac{1}{2}$ hr. with chloroform to give 0.25 g. of a yellow resin.

Of the oil taken, 93 % was thus accounted for. The colourless oil (1.33 g.) yielded by the first ether extraction, on analysis by the Seil method, showed an average total pyrethrin content of 93 % (made up of 81.7, 81.0 % of I and 12.1, 11.3 % of II in duplicate analyses).

The flowers originally taken showed a ratio of pyrethrin I to pyrethrin II of 2.7. This has been changed in the final concentrate to a value of 7. Such a concentrate should be of value for the isolation of pyrethrin I by distillation.

It should be stressed that the quality of the charcoal used plays an important part in the amount needed to obtain a colourless extract. This is clearly seen when the amount used in the preparation of the

colourless extract by petroleum-ether extraction (Table 3) is compared with those that were required in obtaining extracts for the preparation of the pyrethrin concentrates. It was thought possible at one stage that the incorporation of charcoal with the flowers would assist the analytical process, but it was found that the variable nature of the decolorizing charcoal available made the assessment of the amount to be used a matter of some difficulty.

There has been evidence in the work that pyrethrin II tends to be retained by the charcoal more tenaciously than is pyrethrin I, and this fact may be capable of utilization in a further separation of the active principles.

SUMMARY

1. Biological trials have been carried out to determine the efficacy of petroleum ether as solvent for the extraction of pyrethrum flowers for analysis. 95 % of the toxic material was extracted from flowers one year old after only 3 hr. percolation. An extraction period of 8 hr. with petroleum ether is suggested.

2. A method of preparing colourless extracts of pyrethrum and analytical data for such extracts are given. They are shown to be of value for the preparation of concentrates of the pyrethrins. The preparation of a colourless concentrate containing 93 % of total pyrethrins, as determined by a modified Seil method, is described.

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FURTHER OBSERVATIONS ON THE BLOOD COPPER OF NORTHUMBRIAN SHEEP

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(With One Text-figure)

IN a previous communication (Eden, 1939) data were presented on the blood Cu content of 306 sheep, grazing on a Northumbrian hill-side and subjected to five experimental treatments in connexion with the prevention and cure of "border pine". These sheep showed an overall variation in Cu values from 0.016 to 0.164 mg. %, mean 0.088, and although there were slight differences in blood level associated with treatment and with age the general conclusion reached was that moderate increases in the Cu intake did not materially affect the normal blood Cu level. The individual treatments were: A, supplementary feeding with a heavily mineralized cake plus monthly anthelmintic therapy with copper sulphate and nicotine; B, supplementary feeding; C, control; D, control receiving short changes of pasture two or three times a year, and E, anthelmintic treatment. The main experiment on the control of "pine" and on the possible relationship between nutrition and helminth infestation, was undertaken by Mr Lyle Stewart and Dr Ponsford of Newcastle in collaboration with Dr Green and Dr Taylor of Weybridge, and was started in 1936. Facilities were afforded the writer for making an extensive survey of blood Cu data in sheep and since these bleedings were made (February and March 1939) the design of the experiment was materially altered. Changes of treatment and regrouping of certain sheep took place in the autumn of 1939 and the new design of treatments became: A', supplementary feeding as before plus anthelmintic treatment with phenothiazine; B', supplementary feeding; C', control, and E', anthelmintic treatment. As far as the Cu history of the sheep is concerned this involved the abolition of the monthly dosing of groups A and E with 100 c.c. 1% CuSO_4 . Since 1939 (September) the Cu history has been more uniform, groups A' and B' receiving a mineralized cake supplement providing about 12 mg. Cu per day in addition to that of the normal grazing, which is of the order of 14 mg. daily. In June 1940 a

further opportunity for blood studies was given but for various reasons only a proportion of the previous sheep was available for bleeding and unfortunately none of these was from the A group of 1939. Some ninety-four samples were received as well as twenty-four bloods from yearling ewes. The blood samples were taken into Cu-free potassium oxalate and portions of the whole blood analysed for total Cu by a photometric modification of the diethyldithiocarbamate method (Eden & Green, 1940).

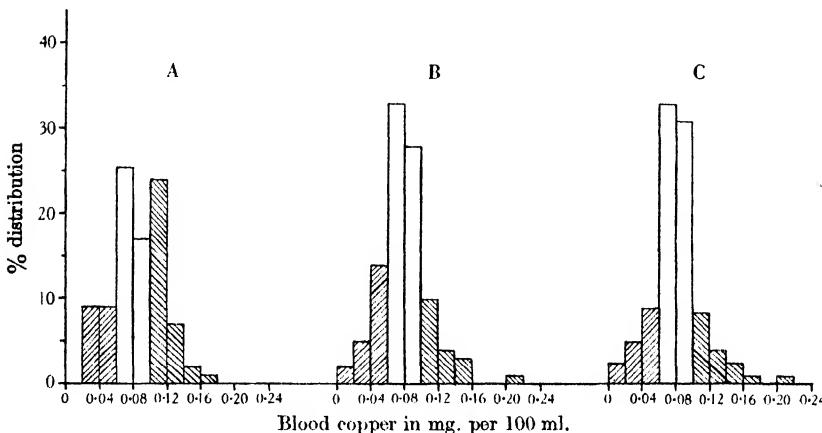


Fig. 1. Percentage distribution of blood copper values in sheep. A, distribution of the ninety-four samples in 1939. B, distribution of the ninety-four samples in 1940. C, distribution of the 118 samples in 1940. Shaded areas denote percentage values falling below 0.06 and above 0.10 mg./100 ml.

In Table 1 are recorded the blood Cu data together with the previous year's figures and previous grouping for comparison. These data are presented in graphical form in Fig. 1 showing, at distribution intervals of 0.002 mg. %, (A) the ninety-four samples of 1939, (B) the corresponding ones of 1940 and (C) these 1940 data combined with those of the twenty-four yearling ewes born in 1939.

The numbers in the individual treatments of 1939 are too small to be treated separately, but the ninety-four sheep considered as a group in that year showed blood Cu values ranging from 0.026 to 0.164 mg. %. 70 % of these values lay between 0.06 and 0.12 mg. %, 19 % were below 0.06 and 11 % were above 0.12. The mean value was 0.086 mg. % compared with the 0.088 mg. % for the whole 306 animals bled at that time. In 1940 these same ninety-four animals ranged from 0.013 to 0.210 mg. %, that is, even wider than the 1939 range despite the greater uniformity of Cu intake, while the mean value was 0.080, slightly lower than before.

The highest figure is that of no. 70, a 1936 ewe, previously one of control group C and now receiving supplementary feeding, whilst of the two lowest values, both in control group C', one was previously on supplementary feeding and the other was a change-of-pasture control. In this group again 70% of the values lay between 0.06 and 0.12 mg. %, 21% were below 0.06 and nearly 9% were above 0.12. Hence the group distribution is much the same as before. Of the ninety-four sheep twenty-two, or nearly a quarter, previously belonged to group E, receiving regular

Table 1. *Blood copper values of Northumbrian sheep. Copper in mg./100 ml.*

Group A'													
Previous group ...	B		C		D		E						
No.	1939	1940	No.	1939	1940	No.	1939	1940	No.	1939	1940		
34	0.117	0.122	65	0.073	0.080	102	0.086	0.077	138	0.078	0.068		
42	0.067	0.074	69	0.029	0.087	103	0.044	0.026	140	0.091	0.055		
43	0.102	0.068	73	0.031	0.108	254	0.073	0.112	267	0.105	0.077		
59	0.097	0.071	93	0.040	0.053	258	0.061	0.080	279	0.108	0.083		
64	0.120	0.071	224	0.085	0.086	259	0.065	0.125	—	—	—		
215	0.105	0.077	226	0.043	0.131	554	0.084	0.083	—	—	—		
309	0.076	0.083	329	0.117	0.089	—	—	—	—	—	—		
314	0.117	0.086	537	0.046	0.077	—	—	—	—	—	—		
Mean	0.100	0.082	Mean	0.058	0.089	Mean	0.076	0.083	Mean	0.096	0.071		
Group B'													
Previous group ...	B		C		D		E						
No.	1939	1940	No.	1939	1940	No.	1939	1940	No.	1939	1940		
47	0.076	0.062	68	0.061	0.084	100	0.114	0.092	131	0.124	0.099		
308	0.085	0.065	70	0.091	0.210	107	0.102	0.086	132	0.138	0.143		
—	—	—	80	0.036	0.105	123	0.088	0.074	141	0.132	0.079		
—	—	—	96	0.053	0.041	255	0.110	0.065	155	0.097	0.077		
—	—	—	328	0.032	0.054	—	—	—	160	0.117	0.083		
—	—	—	533	0.035	0.073	—	—	—	235	0.083	0.118		
—	—	—	—	—	—	—	—	—	272	0.102	0.065		
—	—	—	—	—	—	—	—	—	282	0.164	0.093		
Mean	0.080	0.064	Mean	0.051	0.091	Mean	0.104	0.079	Mean	0.119	0.095		
Group C'													
Previous group ...	B		C		D		E						
No.	1939	1940	No.	1939	1940	No.	1939	1940	No.	1939	1940		
35	0.108	0.068	72	0.064	0.065	106	0.077	0.057	130	0.132	0.122		
37	0.067	0.046	79	0.038	0.111	124	0.064	0.013	137	0.105	0.077		
45	0.100	0.013	89	0.064	0.159	243	0.061	0.033	142	0.102	0.092		
—	—	—	330	0.085	0.038	—	—	—	157	0.117	0.112		
—	—	—	331	0.091	0.160	—	—	—	236	0.152	0.070		
—	—	—	343	0.043	0.038	—	—	—	239	0.085	0.086		
—	—	—	532	0.070	0.068	—	—	—	277	0.094	0.083		
—	—	—	536	0.054	0.095	—	—	—	—	—	—		
Mean	0.092	0.042	Mean	0.064	0.092	Mean	0.067	0.034	Mean	0.112	0.092		

Table 1 (*continued*).

Group E'													
Previous group ...	B		C		D		E						
No.	1939	1940	No.	1939	1940	No.	1939	1940	No.	1939	1940		
33	0.102	0.083	66	0.037	0.052	104	0.072	0.095	129	0.086	0.089		
48	0.140	0.084	67	0.073	0.092	110	0.128	0.084	143	0.102	0.109		
62	0.108	0.044	71	0.117	0.087	127	0.052	0.043	159	0.152	0.070		
63	0.059	0.035	94	0.055	0.115	244	0.070	0.095	—	—	—		
211	0.026	0.043	227	0.105	0.055	—	—	—	—	—	—		
212	0.067	0.047	228	0.064	0.077	—	—	—	—	—	—		
214	0.108	0.102	230	0.064	0.071	—	—	—	—	—	—		
304	0.128	0.087	334	0.070	0.071	—	—	—	—	—	—		
311	0.100	0.092	335	0.067	0.046	—	—	—	—	—	—		
315	0.114	0.089	531	0.076	0.080	—	—	—	—	—	—		
Mean	0.095	0.071	Mean	0.073	0.075	Mean	0.080	0.079	Mean	0.113	0.089		
General mean	0.095	0.070		0.063	0.086		0.082	0.073		0.112	0.089		
Total no. of sheep	23			32			17			22			
1939 lambs													
A'		B'		C'		E'							
No.	Copper	No.	Copper	No.	Copper	No.	Copper						
745	0.089	736	0.092	739	0.065	733	0.074						
753	0.102	743	0.077	740	0.077	742	0.065						
754	0.089	751	0.071	747	0.071	757	0.077						
761	0.131	752	0.083	756	0.030	758	0.065						
777	0.089	759	0.077	764	0.089	766	0.092						
—	—	760	0.092	779	0.007	773	0.092						
—	—	767	0.175	—	—	—	—						
Mean	0.100	Mean	0.095	Mean	0.056	Mean	0.076						
Combined means	0.097						0.067						

treatment with soluble copper so that the cessation of this particular treatment had very little effect upon the average blood Cu level. The evidence available bears out the conclusion previously drawn (Eden, 1939) that the wide variations found between individual sheep in respect of blood Cu level are a natural phenomenon and that in the main these variations are little affected by moderate increases in the Cu intake.

The third chart (C) of Fig. 1 shows the combined values of these ninety-four sheep bled in 1940 with those of the twenty-four yearling ewes. Again the same distribution is found. The mean value of the twenty-four sheep is 0.082 mg. %, but the exceedingly low level of no. 779, 0.007 mg. %, is noteworthy as being one of the lowest blood Cu values on record and certainly the lowest the writer has found in the course of some hundreds of analyses. This small group of yearling ewes shows, apart from three or four animals, a very uniform blood Cu level. However, when groups A' and B' are combined (groups which received

supplementary Cu feeding from birth), the mean blood Cu level of twelve animals is 0.097 mg. %, whilst that of groups C' and E' ("controls" in respect of Cu intake) is only 0.067 mg. %, or, even omitting no. 779 which is exceptionally low, 0.073. In the previous communication it was mentioned that year-old sheep tended to show slightly higher blood Cu levels than older sheep, the difference being more pronounced in animals receiving Cu from birth. Although the general mean of this year-old group of sheep is the same as for the larger group, the blood Cu values of those animals receiving supplementary Cu feeding from birth tends to bear out the previous preliminary indication. Unfortunately, the numbers of animals involved are too small to make the observation conclusive.

In Table 2 are recorded the summarized means of the various sections of the main group of sheep, arranged according to year and to treatment.

Table 2. *Mean blood copper values of sheep arranged in treatment groups. Copper in mg./100 ml.*

Previous treatment	Group ... A'		B'		C'		E'		General mean	
	1939	1940	1939	1940	1939	1940	1939	1940	1939	1940
B	0.100	0.082 (8)	0.080	0.064 (2)	0.092	0.042 (3)	0.095	0.071 (10)	0.095	0.070 (23)
C	0.058	0.089 (8)	0.051	0.091 (6)	0.064	0.092 (8)	0.073	0.075 (10)	0.063	0.086 (32)
D	0.076	0.083 (6)	0.104	0.079 (4)	0.067	0.034 (3)	0.080	0.079 (4)	0.082	0.073 (17)
E	0.096	0.071 (4)	0.119	0.095 (8)	0.112	0.092 (7)	0.113	0.089 (3)	0.112	0.089 (22)
General mean	0.081	0.083 (26)	0.092	0.087 (20)	0.084	0.076 (21)	0.087	0.075 (27)	0.086	0.080 (94)

The numbers in brackets indicate the numbers of sheep that make up the mean in each group.

The general mean of the 1940 values is slightly lower than that of 1939, but the distributions are essentially similar. The mean values for the different group treatments of 1940 are lower than in 1939, except for the control group C of 1939 on all the 1940 treatments, and the 1939 group D receiving A' treatment in 1940. The general rise, from 1939 to 1940, in the thirty-two sheep of group C is quite pronounced. The rearrangement of the groups resulted in the changing round of the sheep on the various hill-side pastures, as well as in change of treatments, and the net effect of the change seems to have slightly elevated the average blood Cu levels. Even considering groups C and D together (previous controls) forty-nine sheep show, on an average, a rise from 1939 to 1940 in contrast to the fall in groups B and E. The general effects all round, however, have been slight, and what small apparent differences exist in the group considered as a whole might conceivably have been smoothed out altogether had it been possible to utilize larger numbers of sheep.

EXTENT OF INDIVIDUAL VARIATIONS

By comparing the extent of the rise or fall of the 1940 figures in relation to those of 1939, an analysis of the extent of individual variations at the two bleedings is obtained. At present little is known of the extent to which the blood Cu level can vary in the same individual. There is no evidence in the literature concerning any rise in blood Cu in ewes during pregnancy as Tompsett & Anderson (1935) have reported for women. If there is any such pronounced rise it might well have been reflected in the data of Table 1, since the 1939 bleedings were made in the later stages of pregnancy whilst those of 1940 were carried out about two months after lambing had occurred. The differences between the individual 1939 and corresponding 1940 blood Cu figures are analysed in Table 3; these differences are grouped at intervals of 0.005 mg. % irrespective of whether they represent a rise or a fall in the previous year's individual values.

Table 3. *Individual variations in blood copper content of sheep. Grouping intervals of 5 µg. above or below the corresponding 1939 value*

Interval (µg.)	0-	6-	11-	16-	21-	26-	31-	36-	41-	46-	Over 50
No. of sheep	13	12	7	10	10	7	5	5	4	4	17
% of total	13.8	12.8	7.4	10.6	10.6	7.4	5.3	5.3	4.3	4.3	18.2

It is seen that over a quarter of the sheep had values in 1940 falling within ± 0.01 mg. % of their 1939 value, but nearly a fifth of the group had values differing by more than ± 0.05 mg. % from their previous values. The general correlation between blood Cu values of the same individual at different bleedings is not very close, and it would appear that the wide variation found between different individuals also occurs within the same individual. Thus no. 45 varied from a 1939 level of 0.100 to 0.013 in 1940, nearly an eightfold difference. So far there has been no reported work on the physiological significance of blood Cu values. Certainly more data on the variation between individuals and within the same individual are required before physiological significance can be established.

Discussion of blood Cu levels in sheep would be incomplete without reference to the work of Bennetts & Chapman (1937), Dunlop & Wells (1938), Dunlop *et al.* (1939), which demonstrated that the feeding of copper sulphate to pregnant ewes considerably reduces the incidence of the disease of lambs known as "enzootic ataxia" in Australia and

“swayback” in England. Bennetts & Chapman reported figures of less than 0.01 mg. % for the blood Cu of four ewes which gave birth to affected lambs, but Innes & Shearer (1940), in a more extensive study in Derbyshire, reached the conclusion that the level of blood Cu in the mother is not the primary factor in determining the incidence of “swayback” in the offspring. Ewes bearing “swayback” lambs in Derbyshire showed an average value of 0.058 mg. % with a range of 0.037–0.070, and ewes on “swayback” farms bearing normal lambs showed a mean value of 0.045 mg. % with a range 0.034–0.061. In non-affected areas higher mean levels of 0.127 and 0.073 mg. % were found, but the range of values, 0.054–0.281 in one case and 0.047–0.127 in the other, precluded close association of incidence of disease with maternal level of blood copper.

Considering the data from apparently normal Northumbrian sheep, seventy-seven animals out of 306 in the previous series (Eden, 1939) and thirty-seven out of 118 in the present series, or approximately a quarter of the flock in both cases, fall below 0.070 mg. %, the upper value for ewes bearing “swayback” lambs in the records of Innes & Shearer. The hill-side pastures in the Northumbrian area concerned have an even lower Cu content (6–10 p.p.m. on the dry matter) than the “swayback” area of Derbyshire. If the disease were a simple question of Cu deficiency reflected in the blood Cu levels of the ewes one would expect a comparable incidence in both areas. Yet “swayback” is unknown on the Northumbrian farm concerned. Although it is beyond the scope of the present paper to comment in further detail on the important economic problem of “swayback”, the findings give general support to the statement of Innes & Shearer that the level of blood Cu in ewes is not the primary factor in determining the incidence of disease in the offspring, despite the beneficial effects of copper administration over the period of pregnancy.

SUMMARY

1. Blood copper data are reported on ninety-four sheep divided into four experimental groups on a “border-pining” hill-side in Northumberland in 1940, in comparison with more extensive findings reported in 1939.
2. The mean value for comparable sheep was 0.080 mg. % in 1940 as compared with 0.086 mg. % in 1939, the overall range, 0.013–0.210 mg. %, being even wider than before.
3. Variations between animals comprising a group were as wide as

between groups, and blood levels were not affected by moderate variations in Cu intake. Only in young sheep was there any evidence that a mineralized cake supplement, containing copper sufficient to double the natural grazing intake, had any elevating effect on blood copper.

4. Variations between values for the same individuals in 1939 and in 1940 were as wide as between different individuals in either year. Over 25 % of the sheep showed Cu levels in 1940 falling within ± 0.01 mg. % of their 1939 values, but nearly 20 % showed figures differing by more than ± 0.05 mg. %, irrespective of differences in group treatments.

5. The significance of the figures for normal Northumbrian ewes is discussed in relation to those reported for Derbyshire ewes bearing lambs affected with "enzootic ataxia" or "swayback".

The author has pleasure in thanking Dr H. H. Green for arranging facilities for this work and Mr C. W. Clarke for valuable technical assistance during its execution.

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THE STORAGE OF ARTIFICIALLY DRIED GRASS

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(With Plate 4 and Seven Text-figures)

INTRODUCTION

THE present paper deals only with the effects of humidity and moisture content on the keeping quality of artificially dried grass, since these are generally recognized to be the most important factors affecting storage deterioration. It should be made clear at the outset that it must be assumed that the grass is satisfactorily dried prior to storage. Practical experience shows that with certain designs of drying plant (particularly where cheap labour is employed) damp patches are occasionally found in the grass at the delivery end of the drier, and that unless these are removed prior to storage, deterioration is inevitable. Examination of bales of dried grass not infrequently reveals the presence of such damp patches, which usually contain from 20 to 40 % of moisture and which are invariably moulded. Obviously no method of storage could overcome casual deterioration from this cause.

As a safeguard against the occurrence of damp patches the tendency of plant designers has been to maintain the grass in contact with the hot drying air for a much longer period than would otherwise be necessary; that is, to allow a considerable safety margin for the drying out of abnormally wet or matted patches of grass. In consequence the moisture content of the bulk of the grass as it leaves the drier is apt to be exceptionally low. Table 1 gives the moisture contents of a typical series of samples taken at random at the delivery end of the drier, where the grass is usually still hot. While the figures show wide variations, it will be seen that more than half of the samples contained less than 5 % of moisture. Moreover, the moisture contents were clearly of a different order from those of samples which had undergone prolonged storage, typical values for the latter being shown in the lower set of figures in Table 1. These represent the moisture contents of random samples which had been ground to a meal, filled into $\frac{1}{2}$ cwt. linen bags, stored in a large corrugated iron shed (partially open on one side) for several weeks, and then transferred to the premises of a large provender merchant. The last

four figures certainly appear exceptionally high, but it may be noted that values of between 14 and 15.5 % have also been found in bales of grass stored in relatively open sheds.

Table 1

Range of moisture contents of random samples taken at delivery end of drier (%):

1.6, 2.0, 2.5, 3.0, 3.3, 3.8, 8.3, 8.5, 9.9, 10.6

Range of moisture contents of random samples taken after prolonged storage (%):

9.6, 10.5, 11.4, 12.2, 13.1, 13.7, 14.4, 14.7, 15.2, 16.4

Determinations of the moisture contents of samples of grass meal taken from the outer and inner layers of bags after storage show clearly that the high values are due to absorption of moisture from the surrounding atmosphere. Typical results are given in Table 2. Bags 4 and 5 show a 3-4 % difference between the moisture content of the outer layer and that of the inner; the latter (i.e. 8-8½ %) may be taken as the initial value of the whole contents of the bag. In bag 3, which was lying on the top of the pile of bags and was therefore more fully exposed to the surrounding atmosphere, absorption has gone a stage further, the inner layer having reached 11 % and the outer layer over 14 % of moisture. These results show, incidentally, the wide range of variations in the moisture contents in different parts of the bags, and re-emphasize the importance of proper sampling in any determination of the mean moisture content.

Table 2. *Moisture content of grass meal in outer and inner layers of bags after storage*

Bag no.	Outer layer	Centre of bag
	%	%
3	14.2	11.6
4	11.2	8.5
5	12.4	8.1

Table 3 shows a more extreme instance of moisture absorption. In this case two bags of dried grass meal, containing initially between 11½ and 12 % moisture, were stored in a shed which was well roofed but of which one side was permanently open to the external air. After 4 months' storage the moisture content of the outer layers of both bags (which were moulded) had reached 20 % and that of the inner layers 15 %. Determination of the mean moisture contents of the bags showed that there had been an over-all increase of 5 %, a figure which was roughly confirmed by actual weighing of the bags. It will be seen that they had gained in weight by from 2½ to 3½ lb. as a result of moisture absorption.

Table 3. *Increase in moisture content of grass meal stored in linen bags in an open shed*

	Bag 1	Bag 2
By moisture determination (%):		
Outer layer (moulded)	19.7	20.0
Intermediate layer	17.2	17.7
Centre of bag	14.9	15.2
Bulk sample, mixed	16.7	16.9
Initial moisture content	11.7	12.0
% increase in moisture content	5.0	4.9
By weighing (lb.):		
Final weight of bag	58.9	58.2
Initial weight of bag	56.6	54.9
Increase in weight	2.3	3.3
% increase in moisture content	3.9	5.7

The conditions under which these bags were stored were, of course, exceptional, but the results were sufficiently striking not only to demonstrate the highly hygroscopic nature of the dried grass, but to justify a more systematic study of the general relationship between storage conditions and moisture content. Moreover, it was felt that, from the practical point of view, such a study was particularly desirable for two reasons: first, because the increased production of dried grass on a farm scale would necessitate the storage of large quantities of the product in farm buildings which have not been primarily designed as feeding-stuffs stores; and secondly, because for farm use it is probable that much of the dried grass will be baled, a form in which it is particularly liable to absorb atmospheric moisture.

PRELIMINARY EXPERIMENTS

It was clear from a study of past work on storage problems that the relationship between atmospheric conditions and moisture content could best be studied by examining the effect of variations in the relative humidity of the surrounding atmosphere, the relative humidity being the most convenient method of expressing the moisture carrying capacity of the air. As a first step, therefore, it was decided to take daily readings of the relative humidity of the atmosphere under widely varying conditions and to relate such readings to the moisture contents of thin layers of dried grass exposed to these conditions. It was hoped by this means to obtain an indication of the ranges of humidity and moisture content which might be expected in farm practice. As regards atmospheric conditions, variations were obtained by maintaining a large room either closed or open to the external air from the late summer to the early

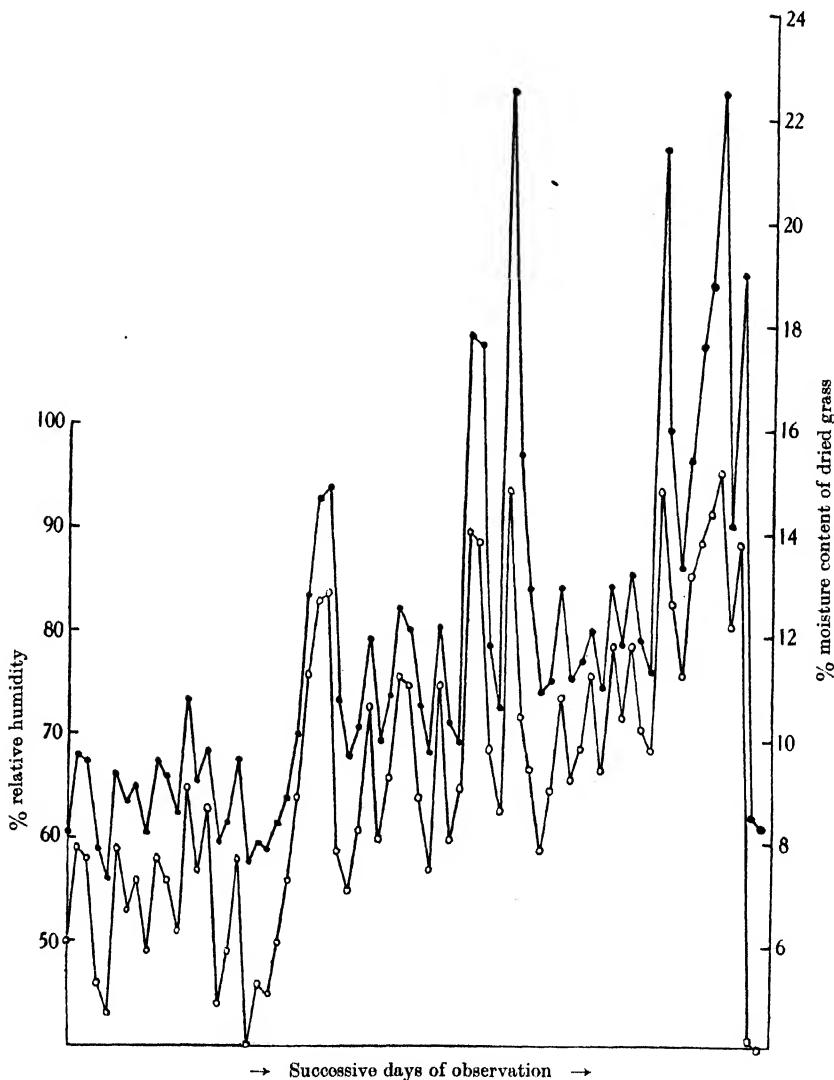


Fig. 1. Daily values of the relative humidity and of the moisture content of a sample of dried grass exposed to varying atmospheric conditions. (Hollow circles, relative humidity; black circles, moisture content.)

winter period, readings of the relative humidity being taken each day. As regards the moisture content, quantities of dried grass meal were weighed out and spread evenly over the surface of Petri dishes, the amount of dry matter in each dish being exactly 1 g. By weighing the dishes daily the variations in moisture content could readily be calculated. The results are given in Fig. 1.

It will be seen that the relative humidities varied from 40 to practically 100 %, which may be taken to represent the extreme range of variation between a dry warm room with closed doors and a cold room freely open to the external atmosphere on a rainy day. The striking points illustrated in Fig. 1 are, first, the extraordinarily close parallelism between the humidity and the moisture content of the grass, and secondly, the very high moisture contents attained at high humidities, the extreme values reaching over 22 % of moisture when the relative humidity exceeded 90 %. These figures appeared all the more striking when it was realized that they might not represent equilibrium conditions, since the dried grass layers would probably have been exposed to the high humidities for only a few hours. In Fig. 2 the data for Fig. 1 have been plotted in the form of a scatter diagram in which the moisture contents are compared directly with the relative humidities. The points fall fairly closely on a smooth curve, demonstrating conclusively that there is a definite relationship between moisture content and humidity.

RELATION BETWEEN RELATIVE HUMIDITY AND MOISTURE CONTENT

The next step was to determine more accurately the exact nature of this relationship. For this purpose a series of dried grass meal samples were exposed in thin layers in Petri dishes to atmospheres in which the relative humidity was accurately controlled. In practice the dishes were placed in a series of desiccators containing solutions of sulphuric acid of varying concentrations, each concentration corresponding to a definite relative humidity. The humidities used were 100 (i.e. pure water), 90, 85, 80, 74, 67, 60 and 40 %. It will be seen that these cover the entire range shown in Fig. 2. The dishes were weighed daily until equilibrium was established or until mould growth interfered with the moisture absorption. A typical series of results is shown in Fig. 3.

It will be seen that the relationship between relative humidity and moisture content is represented by a series of smooth curves. It is clear, however, that equilibrium is not normally established for several days, particularly at the high humidities, though it may be noted that (as

might be expected) the rate of establishment of equilibrium was found to depend on the exact conditions under which the experiment was carried out—for example, on the number of dishes in the desiccators, the surface

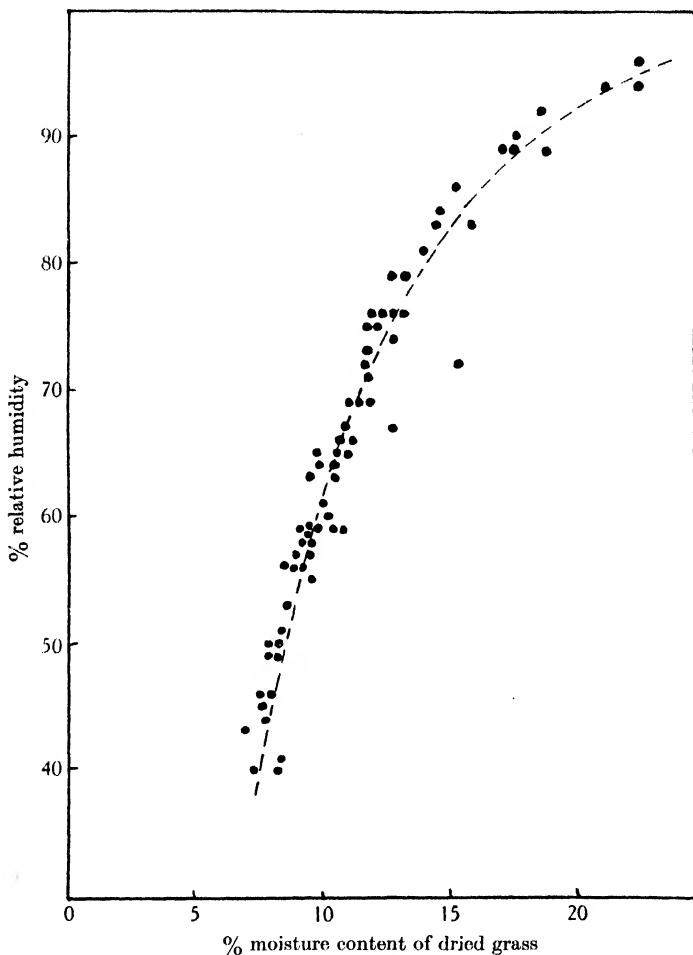


Fig. 2. Relation between the relative humidity and moisture content of a sample of dried grass exposed to varying atmospheric conditions.

area of the sulphuric acid, and similar factors. The outstanding fact shown in Fig. 3 is, however, the very high moisture contents of the grass when equilibrium was attained in atmospheres of high humidity. Thus at 80 % humidity the moisture content was roughly 18 %, at 90 % humidity it was 28 %, while at 100 % humidity it reached the exceptionally

high figure of 50 %—that is to say, the dried grass had been able to absorb its own weight of moisture.

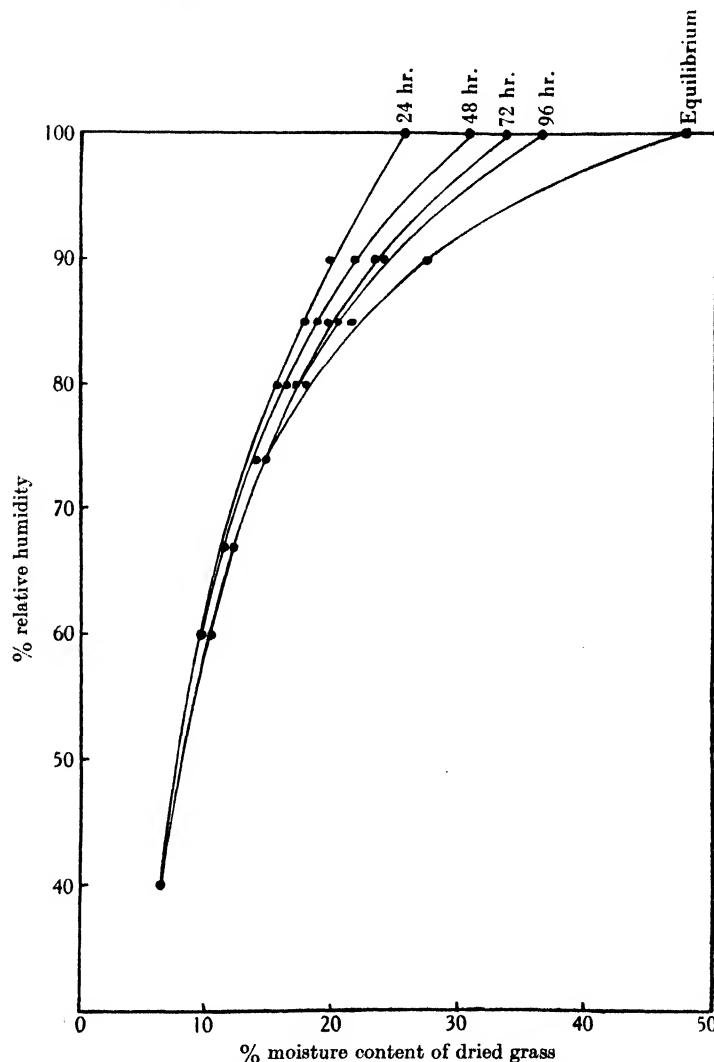


Fig. 3. Rate of establishment of equilibrium between relative humidity and moisture content.

These results have been repeated with a number of different samples of dried grass, in order to determine whether differences in composition affect the humidity-moisture relationship. Fig. 4 shows the results for

four samples which may be taken as typical. The protein contents of these samples varied from 10 to 20 %, so that the samples represent the extremes of quality likely to be encountered under practical conditions.

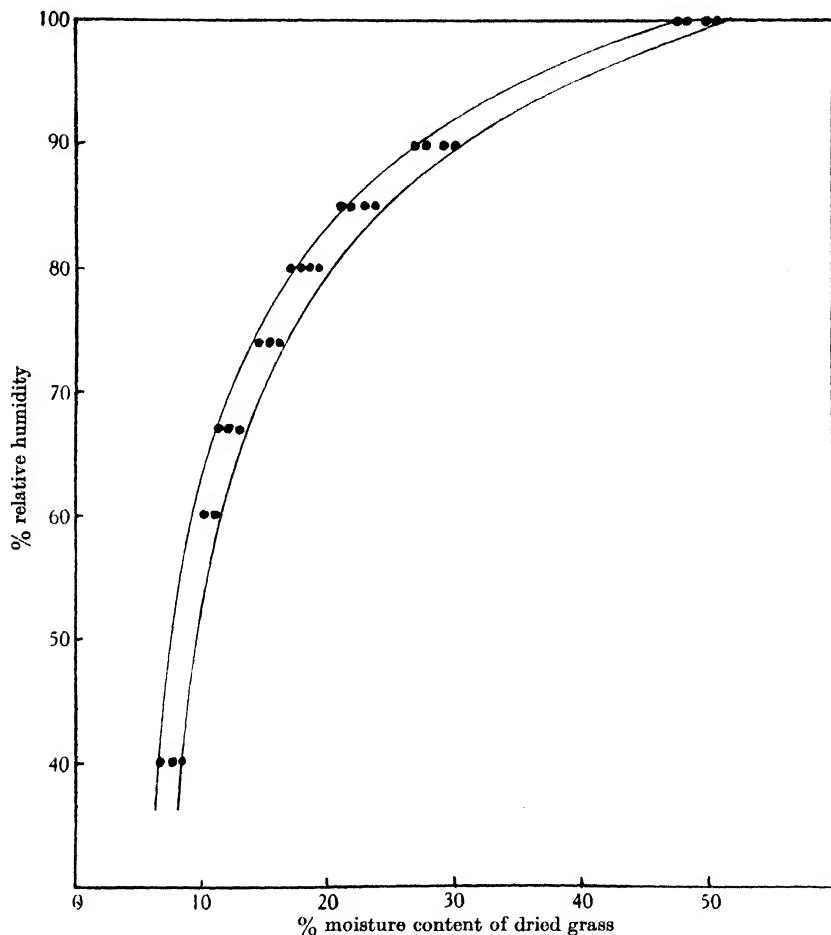


Fig. 4. Relation between relative humidity and moisture content at equilibrium (four samples).

It will be seen that, although the individual points show some variation, in general they fall along a fairly narrow band which coincides closely with the equilibrium curve plotted in Fig. 3.

The significance of these results will at once be apparent. The moisture content of freshly dried grass, which may be taken as not more

than 8 %, corresponds to a relative humidity of 40 %. Such a low humidity is seldom recorded in the open air under British climatic conditions. According to the *Meteorological Office Tables of Humidity* the average summer figure for Great Britain is between 60 and 70 %, while the winter figure is generally between 80 and 95 %. These ranges of humidity correspond to moisture contents in the dried grass of between 10 and 12 % in the summer and 18 and 30 % in the winter. In view of these facts the figures quoted earlier in this paper regarding the increases in the moisture content of sacks of grass meal during storage are not surprising: it is, indeed, clear that unless the storage rooms are kept closed to the external air, considerable moisture absorption is always inevitable. In this connexion it is interesting to note that if a pile of dried grass is left overnight in an open shed, it is usually found to be too moist by the morning to be satisfactorily ground in an ordinary "swing-hammer" mill.

OBSERVATIONS ON MOULD GROWTH

The next step was to determine the level of humidity and moisture content at which deterioration would be likely to develop. It was felt that useful preliminary information on this point would be most easily obtained from a study of the mould growth which takes place in thin layers of dried grass exposed to controlled atmospheres. Dishes of dried grass were, therefore, stored in desiccators of varying humidities for periods up to 18 months and were examined microscopically for visible mould growth (using a low-power objective) at appropriate intervals. Rough trials showed that the critical humidity for mould growth lies between 67 and 80 %. A large number of desiccators were therefore used over this range, i.e. 67, 70, 72, 74, 76, 78 and 80 %. Typical results are shown in Fig. 5.

It was found that two definite stages of mould growth could be readily differentiated, namely, (i) formation of mycelium, visible at first as single threads on the surface of the grass and ultimately proliferating to form a "cobweb" structure throughout the mass of the material, and (ii) fructification, shown by the formation of sporangia, conidiophores and perithecia. Since the moulds present were of mixed types, the actual appearance of a field was found to vary from sample to sample, though at the lower humidities the flora tended to be limited to a very few types, notably of the *Aspergillus glaucus* group. Typical fields are shown in Pl. 4.

The competition of small weeds produced significant decreases both in yield and in percentage of ware, as is shown by the comparison between treatments N and N'. The actual percentage reduction of yield on the N plots below the mean of the other five treatments for each of the six blocks was:

Block	...	I	II	III	IV	V	VI
% reduction in yield		11.3	18.4	27.1	23.5	43.4	0.9
% reduction in ware		2.2	-1.4	10.7	12.6	13.6	2.3

Only on the plot in block V was the weed infestation really severe, the weeds being principally bind-weed (*Convolvulus avensis*), groundsel (*Senecio vulgaris*) and young goose-foot or fat-hen (*Chenopodium album*). The reduction of yield by nearly one-half on the weedy plot of block V would not have been surprising if the plot had remained weedy throughout the season, but it was thoroughly cleaned twice before ridging-up on 6 July, and it remained clean thereafter. The deleterious effect of the weeds in the early stages of the crop growth, before the cultivations were given, must therefore have accounted for most of the yield reduction. Substantial reductions also resulted on four of the remaining five plots on which, judged by eye, the weeds would not appear capable of harming the plants. There were indications, discussed below, that competition for moisture was the principal reason for weed damage.

Tubers greened by exposure to light. All tubers showing signs of greening were carefully picked out and weighed separately for each plot:

Treatment	F	F'	N	N'	U	U'
% of greened tubers	0.59	0.52	0.88	0.43	1.46	3.25
Means for cultivations	0.56 % (bouted)	0.65 % (bouted)	0.65 % (bouted)	0.43 % (flat)	2.35 % (flat)	

Although the plots grown on the flat produced a relatively high proportion of greened tubers, the slight cover provided by scraping (U) with flat hoes reduced this wastage to less than 1.5 %. This is still nearly three times the proportion wasted by the bouted-up plots on which little more than half of 1 % were spoiled. It might be further reduced if the scraping sweeps were designed to cast more soil sideways.

THE 1939 EXPERIMENT

The field trials for two consecutive years had shown no effective response by the potato crop to ridging-up, with or without frequent grubbing, when all weeds were removed by other means. Practical growers, with whom the results were discussed, suggested that "It takes deep working to do any good". The depth of 3 in., previously used as

Size of tubers.

All potatoes were lifted by hand and passed over a $1\frac{1}{2}$ in. riddle. The weight of tubers affected by sunburning or greening was also noted for each plot.

The results of this experiment are given in Table 2.

Table 2. *Total yields and percentage ware for 1938 experiment*

Treatment symbol	Total yield (expressed as tons per acre)						Standard errors
	F	F'	N	N'	U	U'	
No. of cultivations	6	6	2	2	0	0	
Details of cultivations	Given early	Given later	Some weeds	Weeds hand-picked	Surface scraped $\frac{1}{2}$ in.	Weeds hand-picked	
Total yields in tons per acre	8.94	8.70	6.83	8.80	8.76	8.49	± 0.44
Mean for six cultivations	8.82						
mean for no cultivations			8.63				
difference	0.19	± 0.31					
difference due to weeds, N'-N			1.96	± 0.63			
Ware % of total yield	92.7	92.4	84.9	92.1	92.5	90.4	± 0.79
Differences: N'-N = 7.2 (significant); U-U' = 2.1 (not significant)							± 1.12

The most striking feature of these results is that the means for the four weed-free, motor-hoed treatments F, F', N', and U showed no apparent response by the crop to the three very different tillage treatments. The differences in the means, both of yield and of percentage ware, are not only well within the margin of error, but are also, from the viewpoint of the practical grower, negligible in comparison with the differences in the cost of cultivation. Of the six comparisons between F and U cultivations in the six blocks three favour each treatment. The almost identical mean yields of the N and U plots show that, in the absence of weeds, two grubblings and a ridging-up of the plants served no purpose other than the protection of the tubers by ridging. The ridges do not thus appear to have provided an environment more encouraging to the crop than the flat culture of the plots receiving three surface scrapings only. The four extra grubblings produced little or no effect, although there is a suggestion of a slight response in yield when these are given earlier; but the difference of 2.8 % in yield between F and F' is not statistically established, and may be due to chance.

The use of flat sweeps on the motor-hoe for removing weeds at the surface level proved to be a better method than the hand-picking. The necessity of hand-weeding when the soil was too wet, in the early part of the season, caused excessive packing, in spite of the fact that long boards were laid between the rows on these occasions in order to minimize trampling.

cultivations (F) beginning 14 days and ending 19 days before the other (F'). The "N" or simple grubbing-and-ridging treatment was duplicated in order to estimate separately the effects of the ridging and weed competition. On one set of plots (N) the weeds were controlled by two cultivations only, the plants being otherwise left to smother the weeds, while in the duplicate set (N'), given the same tillage, all weeds were removed with a minimum of soil disturbance, by careful hand-cleaning. The control treatment was also duplicated, in an attempt to secure weed destruction with minimum soil disturbance, by a more practical means than hand-picking. The experiment was laid down on a contiguous strip of the field used for the 1937 trials.

Plan of experiment.

A 6×6 Latin square was used, all plots being cropped with Doone Star potatoes.

Details of cultivation treatments.

F. Six cultivations, the last one being followed immediately by bouting-up.

F'. Six cultivations given from 15 to 19 days later than those for the "F" plots, and finally bouting-up.

N. Two cultivations given when necessary for weed destruction. The second was followed by bouting-up.

N'. Cultivations as for "N" plots, but weeds eliminated by hand-picking.

U. No mulching, but the weeds were removed by surface scraping with flat sweeps on the motor-hoe set by a trailing wheel to a depth of about $\frac{1}{2}$ in.

U'. No cultivations. The plots were kept completely free from weeds by hand-picking.

All plots were hand-hoed lightly between the plants before bouting-up.

Trampling of the soil was avoided during hand-picking of weeds by the use of long light boards.

Size of plots.

Approximately $\frac{1}{100}$ acre.

Planting details.

Four rows constituted a plot, of which only the middle two were measured. Rows were set 36 in. apart in order to minimize haulm damage from cultivations. Plant intervals were reduced to 1 ft. to compensate for increased row width.

Table 1. *Total yield and percentage ware of potatoes (1937)*

(a). Effect of frequency of inter-row cultivation

No. of grubblings	None	Two	Four	Standard error
Total yield in tons/acre	12.33	10.37	12.36	±0.595
Increase		-1.93	+1.99	±0.842
% ware	91.79	88.11	89.80	±0.88
Increase		-3.68	+1.69	±1.24

(b) Effect of subsoiling

	Shallow ploughing without subsoiling	Shallow ploughing with subsoiling	Difference due to subsoiling	Standard error
Total yield	12.23	11.10	-1.13	±1.78
% ware	90.87	88.93	-1.94	±2.51

total yield where the weeds were checked by two grubblings only. These grubblings, given at times when the weeds could best be destroyed, prevented them from establishing any obvious appearance of competition with the potato haulm. This suggests that even comparatively small weeds, growing thickly, can compete seriously with the potato crop. The weed effects thus disguised any possible effect of ridging-up on yield.

The subsoil ploughing had no effect on the crop yield or size of tubers. It is difficult indeed to imagine how it could be expected to benefit the crop on such a well-drained sandy soil, unless the sand were too compact to permit root penetration. In digging the pits for water-table location, however, roots under normal ploughing were observed well below the 12 in. level reached by the subsoiler. It must be considered probable that the subsoiling, if fairly and critically tested, would have proved to be equally ineffective on the similar neighbouring soils in which this identical equipment had been working for some years.

THE 1938 EXPERIMENT

The failure of the potato crop to respond to inter-row grubbing and ridging in 1937 was either an unusual lapse from the characteristics traditionally ascribed to the crop, or else it was an indication that the potato is not in reality sensitive to intensive tillage under these conditions of soil and climate. The results could only be interpreted therefore in the light of further trials, and the main comparisons were therefore repeated in the design for the 1938 experiments.

The intensive cultivations in 1937 had continued rather late in the growth stage of the plant. To investigate the manner in which this might affect the results, the "F" treatment was duplicated in 1938, one set of

THE 1937 EXPERIMENT

The same area as used in 1936 received autumn cleaning after the potatoes, but a wet spring delayed ploughing in 1937 until 8 April. Although the subsoiling had shown no effect in 1936, it was an operation firmly believed in by neighbouring farmers, so that three of the six columns of experimental plots were subsoiled.

Plan of experiment.

Thirty-six plots, arranged in a special Latin square of which three rows, selected at random, were subsoiled.

Half of the plots carried "Majestic" potatoes while the remaining half were uncropped.

Three cultivation treatments were carried out.

Cultivation treatments.

F. Four cultivations; a loose 3 in. mulch was maintained, and earthing-up was thorough.

N. Two cultivations; given to destroy weeds.

U. No cultivations; weeds controlled by careful hand-picking, supplemented by weed-killer on the blank plots.

Ploughing treatments.

S. Shallow ploughing-in of farmyard manure, 5 in. only.

D. Subsoiling a further 9 in.; otherwise as for S.

Details of planting.

The plots were approximately $\frac{1}{100}$ acre. Potato plots carried four rows 32 in. apart, of which only the two centre rows were weighed.

Size of tubers.

All the potatoes were passed over a $1\frac{1}{2}$ in. riddle, and the chats and wares weighed separately for each plot.

The yields of the different treatments are given in Table 1.

The plots four times grubbed and then ridged up showed no difference, as compared with the uncultivated weed-free control plots, in total yield or percentage ware. Thus the inter-row cultivations appear to have benefited the crop only by destroying the weeds and not by maintaining a loose tilth.

The importance of this weed destruction is indicated by the small but significant decrease in the percentage of ware tubers, and the suggestion, although only on the 7 % level of significance, of a larger decrease in the

one end of the area to about 10 ft. at the other, and in the first two years the effect of the depth of the water-table on crop yield was measured.

All the grubbing cultivations were carried out with an "Auto-culto" motor hoe. Three "duck-foot" tines were used for the earlier grubblings and a central one with a narrow plain chisel point on either side for the later ones. These chisel point tines were moved in towards the centre of the row as the season progressed. Guards of bent iron were fitted over parts of the hoe to avoid haulm damage in the later operations.

In 1938 and 1939 the motor hoe was fitted with a device for keeping the weeds down without mulching the surface soil. The device consisted of three sweeps which scraped the surface soil without loosening it appreciably. The centre sweep was V-shaped and 12 in. across, while the side sweeps were set to work as near as possible to the plant. These two outer sweeps moved the shallow layer of earth loosened from the surface to the sides of the rows where it helped to protect the tubers from sun-burn. At first a trailing wheel was fitted to ensure that the sweeps did not disturb more than the first $\frac{1}{2}$ in. of the soil surface, but this was later discarded as the operator found no difficulty in keeping them on the surface.

The experiments described here were all carried out at Ottershaw Park, Surrey. The soil is formed from the Bagshot Sands and contains up to 19 % clay in the top foot, much less in the second foot, and mainly sand and gravel below that. The field in which the experiments were carried out had been old pasture ley which was ploughed up in 1935 and sown to kale which failed. The area had become extremely foul with creeping thistle and other weeds when the land was laid down to this 4-year series of experiments.

The 1936 experiment was purely preliminary and was laid down with the dual object of cleaning the ground and of evolving a suitable technique for the cultivation experiments. Two ploughings were given, and with the second subsoiling was carried out across strips of the field. Only one-third of the plots was cropped. Three cultivation treatments, five grubblings, one grubbing and a hand-weeded control treatment were given. In the very wet growing season of 1936, however, the thistles got out of hand, and the latter two series of plots had to be cleared at intervals, instead of being kept continuously clean. They gave approximately equal yields while the plots kept clean by five grubblings showed a 14 % increase. It was not possible to ascertain how far this was due to weed destruction as compared with other factors. There was no sign of response to the subsoiling.

STUDIES IN SOIL CULTIVATION

IX. THE EFFECT OF INTER-ROW TILLAGE ON THE YIELD OF POTATOES

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(With Two Text-figures)

THE inter-row tillage of a growing crop forms an intermediate part of a complex series of cultural operations. It is conditioned both by the efficiency of the seed-bed preparation and planting and by the need to secure favourable conditions for harvesting and for the subsequent crops of a rotation. Further, much confusion exists between the cultivation practices of successful growers and the various attempted explanations for these practices. The author has given elsewhere (1941) a general discussion of the published experiments on the effect of inter-row tillage on various crops. The experiments described in this paper were designed primarily to see if the main object of the inter-row tillage of the potato crop was weed suppression, or if the fine loose mulch which was produced as a by-product of this method of weed control had any beneficial effect on the growth of the crop.

Three primary comparisons were made in these experiments. In one the weeds were removed without any appreciable mulch being produced, either by hand-pulling or by a very shallow scraping of the surface soil. In the second, the weeds were destroyed by repeatedly grubbing in between the rows, thus maintaining a fine tilth. The third followed the usual farming practice of the neighbourhood, which consisted of a preliminary grubbing at an early date and then a second grubbing followed immediately by the earthing-up of the plants. The main object of the second grubbing was to obtain sufficient loose soil for the bouting plough to form good ridges easily.

A second object of the experiments was to find the effect of surface mulching on the moisture content of the soil, but owing to pressure of work this could only be investigated in 1937, the first year of the full experiments. In the course of this investigation, however, the depth of the water-table was found to fall from an average depth of 3 ft. 6 in. at

absence of mould growth. It is clear that visible mould growth (although in four instances such growth was very sparse) is a prerequisite to the development of musty smell.

SUMMARY

1. Experiments have been carried out to determine the relation between the moisture content of artificially dried grass and the relative humidity of the surrounding atmosphere. This relation is given by a smooth curve. At relative humidities which are typical of outdoor winter conditions in the British Isles (80–90 %) the equilibrium moisture content lies between 18 and 30 %.

2. The rate of moulding is directly related to the relative humidity and therefore to the moisture content of the dried grass. For reasonably safe storage a relative humidity of 67 % should not be exceeded. This corresponds to a moisture content of roughly 13 %. Even this low value does not confer absolute immunity from mould growth.

3. The importance of extending storage trials over long periods is stressed. In one instance storage for 300 days was required before mould growth occurred. Neglect of this fact may account for the widely held view that a relative humidity of 70–75 % provides safe storage conditions.

4. Attempts to detect mould growth at an early stage by plating methods proved unsuccessful. It is shown that the two earliest and most reliable indications of mould growth are (i) the appearance of visible mycelium when the product is examined under a low-power objective, and (ii) the detection of a musty smell.

The author desires to acknowledge his indebtedness to Dr A. A. Nichols and Dr C. Higginbottom, who carried out the plate counts, and to Miss M. H. G. Crichton who undertook the examination of the samples of grass meal used in the long period storage trials.

(Received 10 October 1940)

Table 6. *Relation between mould growth and mustiness in samples of dried grass stored for 2 years**

Smell	Considerable growth of mould mycelium	Very sparse growth of mould mycelium	No mould growth
Musty	12.2 [†] , 13.0, 13.5, 13.5, 15.9, 17.9, 18.1, 19.2, 19.2, 19.7, 20.0, 20.1, 21.6, 21.7, 23.8	11.0, 12.2, 13.0, 15.0	—
	Mean 17.96; s.d. 3.44	Mean 12.81; s.d. 1.46	
Normal	12.0, 13.0, 13.7	11.0, 11.0, 11.2, 11.4, 11.5, 12.2, 12.4, 12.5, 14.3, 14.3, 14.5	9.6, 9.7, 10.0, 10.5, 10.7, 10.8, 12.7, 13.5, 14.2, 14.6, 14.9, 16.2
	Mean 12.9; s.d. 0.69	Mean 12.62; s.d. 1.52	Mean 12.28; s.d. 2.25

* Samples with abnormal off-smells are omitted from this table.

† These figures represent the moisture contents of the individual samples.

bottle, being sometimes at the bottom, sometimes on the sides, and sometimes on the upper surface of the grass meal. Thirdly, it is almost certain that the mould flora would vary from sample to sample, and this might well account for the variability in the mould development in individual samples. This explanation receives strong support from a study of the detailed records of the samples. Such a study shows that each set of four subsamples usually exhibited a very similar type of growth. For example, three of the subsamples of grass meal D showed mould mycelium at 12.2–12.5 % moisture content, while the three equivalent subsamples of grass meal B showed no growth or mustiness at 14.2–14.3 % moisture content. Again, all the subsamples of grass meal L gave either a heated or fruity smell (moisture content 13.2–14.0 %), while the subsamples of grass meal G gave a pungent or fermented smell (moisture content 16.4–18.4 %) but no mustiness. These results could be most readily explained on the basis of a similarity in the flora within the subsamples, and of differences in the flora between the various grass meals. Whatever the explanation, the results recorded in Tables 5 and 6 demonstrate in a striking way the need for caution in attempting to differentiate the limits of moisture content which can be safely recommended for long period storage.

One further point may be noted. It has already been stated that the detection of mould mycelium constitutes the most delicate test for mould growth, and that mustiness only develops at a later stage of deterioration. This fact is clearly illustrated in Table 6. It will be seen that in fourteen instances mould mycelium was detected in samples which gave no musty smell: on the other hand, in no instance was mustiness detected in the

Table 5. *Condition of dried grass samples after storage for 2 years*

Mould growth	No. of samples	Mean moisture content %	S.D. %	Range of extreme values %
No mould growth	12	12.03	2.25	9.6-16.2
Doubtful traces of mycelium	7	13.33	1.14	11.2-15.2
Sparse mycelium	18	13.06	1.59	11.0-17.0
Fine network of mycelium	8	13.40	2.53	11.7-19.2
Fairly vigorous mycelium growth	8	14.90	2.23	13.0-20.0
Mould overgrowth	11	19.71	2.00	16.8-23.8
Smell				
Normal	29	12.38	2.59	9.6-16.2
Heated, pungent or stale	9	13.80	1.89	11.6-17.0
Fruity or fermented	6	15.88	2.41	13.2-19.6
Musty	20	16.91	3.61	12.2-23.8

As regards mould growth, it will be seen from the upper half of the table that the *mean* moisture content bears a close relationship to the degree of development of the mould growth, and that at *mean* moisture contents above 13 % mould growth is liable to take place. In general the same finding applies to the development of abnormal smells in the stored product, details of which are given in the lower half of Table 5. The standard deviations and still more the ranges of extreme values show, however, that there are marked variations in the moisture limits for mould growth *when individual samples are considered*. For instance (to take an exceptional case) one sample containing 16.2 % moisture entirely failed to show any mould development or to produce mustiness, while another containing 12.2 % gave a definite musty smell and was found on examination to be interspersed with a fine network of mould mycelium.

This fact is shown more clearly in Table 6, where the relationship between mould growth and mustiness is set out in a sixfold table. It will be seen that twelve samples with moisture contents below 13 % showed mould growth and/or mustiness, while five samples with moisture contents above 13 % showed no deterioration on storage.

Three explanations might be offered to account for this observation. First, it is possible that the samples concerned were somewhat less hygroscopic than normal. Since mould growth is primarily a function of humidity, such samples would exhibit mould growth at abnormally low moisture contents. Secondly, it is conceivable, though unlikely, that mould growth occurred because of localized changes in the moisture content (and therefore in the humidity of the surrounding air) in different parts of the storage bottle. This view receives some support from the fact that mould growth was occasionally found to be localized in one particular area of the material. The area varied, however, from bottle to

One further fact re-emphasizes the danger of relying on plate counts for early indications of mould growth. It will be seen from Fig. 6 that in most samples there was a definite fall in the plate count during the initial period of storage. This might well be taken to indicate that the microflora were dying out and that subsequent deterioration would not therefore occur. Fig. 6 shows, however, that this reduction was only a transitory phase, and that it was subsequently followed by a marked multiplication of the surviving moulds. As an extreme example one may take the sample containing 16.5 % moisture, the plate count of which had fallen at the end of 40 days from 9000 to 300 per g. of grass. Yet by the 70th day it had risen to 95,000 and by the 83rd day to nearly 8,000,000 per g. This observation clearly indicates the serious limitations of the plate count as a test of storage conditions, and incidentally demonstrates once again the necessity for basing conclusions only on long period experiments.

OCCURRENCE OF MOULD GROWTH ON PROLONGED STORAGE

The results detailed above have shown that on very prolonged storage mould growth may take place at relative humidities (and consequently at moisture contents) very much lower than had hitherto been reported. Although under practical farm conditions the period of storage would be unlikely to exceed 6-9 months, it appeared desirable to obtain further information regarding the occurrence of mould growth on dried grass stored for a much longer period.

For this purpose a series of sixteen samples of dried grass were stored for a period of 2 years. Prior to storage portions of each sample were sieved into three fractions, containing respectively the coarse, medium and fine particles of the grass meal. The original and sieved fractions therefore comprised a total of sixty-four subsamples. The advantage of this procedure was that each set of four subsamples was derived from the same bulk of dried grass (and was therefore presumably contaminated with the same mould flora), but possessed somewhat different contents of nutrients¹ and probably appreciably different moisture-humidity relationships. All the samples were stored in stoppered bottles in the dark and at room temperature. At the end of the 2-year period the samples were examined for visible mould growth with a low power objective and were also tested for abnormal or musty smell. Table 5 gives a summary of the results.

¹ Recent experiments show, for instance, that the protein contents of the coarse and fine fractions of dried grass meal may vary by as much as 60-100 %.

Kilner jars. The other has been taken from Fig. 5, and represents the relation between moisture content and the first appearance of mycelium formation during storage in desiccators at room temperature. Since Fig. 5 was based on humidity measurements, the corresponding values of the latter have been inserted in the right-hand margin of Fig. 7.

It will be seen that, although all three curves are of the same general shape, those representing mycelium formation and musty smell fall well below the plate count curve. In fact, bearing in mind that the data for mycelium formation were obtained at room temperature and that the

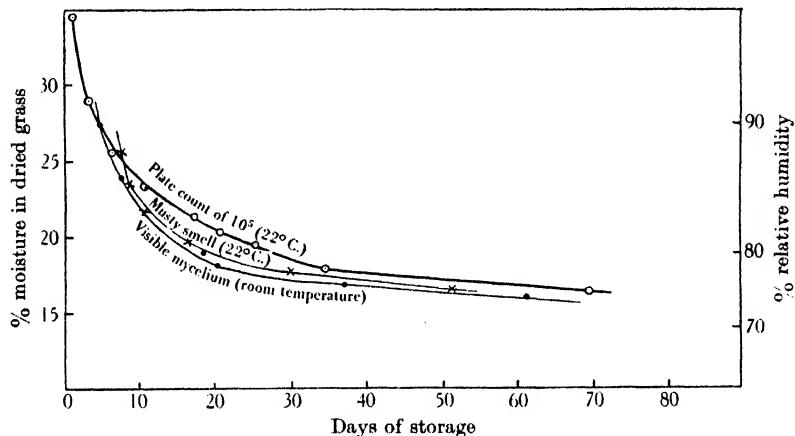


Fig. 7. Relation between formation of visible mycelium, development of musty smell, and increase in mould counts during the storage of samples of dried grass of various moisture contents.

other two curves refer to conditions of accelerated storage at 22° C., the observations fall into logical order. Thus at any given moisture content mycelium formation is invariably seen to precede the detection of musty smell, while the latter in turn is capable of detection long before the plate count has shown a significant increase.¹ For example, in a sample with a moisture content of 17½ %, mycelium formation was visible at the 29th day, a musty smell was detectable about the 35th day, while a plate count of 10^5 was reached only after some 47 days of storage. It is clear from this fact that plate counts are of little value as a means of detecting deterioration at an early stage. If microscopic examination for mycelium is impracticable, the next best test is the detection of mustiness by smell.

¹ This statement does not apply to samples of high moisture content (above 25 %), where mould growth is so rapid that consistent observations are impracticable.

STORAGE EXPERIMENTS WITH DAMPED GRASS

Samples of 200 g. of dried grass were damped with varying quantities of water, using a fine jet atomizer, so as to produce moisture contents in the well mixed material of from 13 to 35 %. The treated samples were stored in closed Kilner jars at 22° C., this temperature being employed in order to accelerate mould growth. Portions equivalent to 1 g. of the original dried grass were removed from time to time from each of the Kilner jars and were examined for mould development by plating out on beerwort agar as described in the previous section. The results of a typical series of tests are shown diagrammatically in Fig. 6, where the plate counts for each moisture content are plotted on a logarithmic scale against the period of storage.

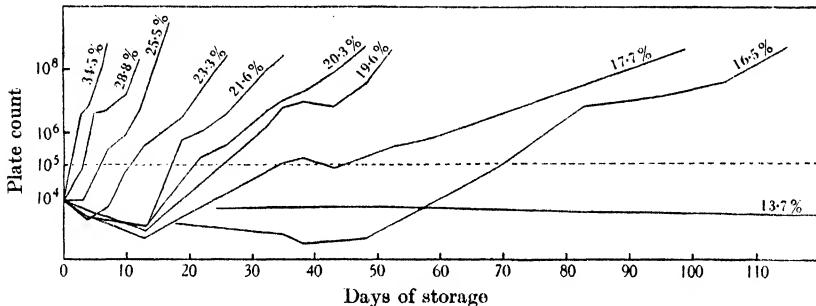


Fig. 6. Increase in mould counts during the storage of samples of dried grass of various moisture contents (storage temperature 22° C.).

It will be seen that the counts tended to increase progressively during storage, but that the rate of increase was dependent on the moisture content. Thus the sample containing 34.5 % moisture showed an increase of the plate count into millions within 4-5 days, with 20-22 % moisture a similar increase required 2-5 weeks, while with 16.5 % moisture the time taken was 10-12 weeks. No increase was found in the count of the sample containing 13.7 % even after 154 days. The regularity of these differences is seen more clearly in Fig. 7, where the points corresponding to plate counts of 10^6 have been transferred from Fig. 6 and plotted so as to relate directly the moisture content and the period of storage. It will be seen that the points fall uniformly on a smooth curve.

In order to determine how far such plate counts might be of value as an early indication of mould growth, two further curves have been inserted in Fig. 7. One of these represents the results of observations which were made on the occurrence of musty smell in the contents of the

mould growth would be proceeding normally. The results of a typical series of experiments are shown in Table 4.

Table 4. *Relation of plate counts to mould growth*

(Original plate counts: bacteria 23,800; moulds 440)

Sample	Relative humidity	Approximate moisture content	First appearance of mould mycelium				Appearance of general fructification (x days)				Final examination ($x + \frac{1}{2}x$ days)	
			Days stored	Bacteria	Moulds	Days stored	Bacteria	Moulds	Days stored	Moulds*		
H	85	24	8	18,050	400	13	13,000	850,000	19	19,000,000,000		
G	80	19	18	17,700	100	25	8,200	1,130,000	37	325,000,000		
F	78	18	21	17,450	300	37	17,400	100,000	56	11,300,000,000		
E	76	17	37	15,000	650	56	18,000	30,000	89	1,080,000,000		
D	74	16	61	18,100	450	98	10,000	124,000	147	100,000,000		
C	72	15	133	3,700	200	230	12,000	44,400	520	380,000		
B	70	14	303	5,800	100	No fructification at 520 days			520	900		
A	67	13	No mycelium at 520 days†	No fructification at 520 days					520	—		

* Bacterial count impracticable owing to overgrowth.

† One small focus of mycelium on one out of four plates.

These results show clearly that during the initial period of mould growth, i.e. when only mould mycelium is visible, growth is solely vegetative: no increase occurs in the mould count. On the other hand fructification is, as would be expected, accompanied by a marked increase in the mould count, while at the final examination the enormous multiplication in the number of mould spores indicates that by this time growth is proceeding normally. It must be concluded from these results that mould growth cannot be detected at an earlier stage by plating than by direct microscopic examination for mould mycelium: in fact the latter not only constitutes an earlier criterion of deterioration but provides at the lower humidities an altogether more delicate indication as to whether such deterioration will ultimately occur. Nevertheless, it appeared that the plating method might be of value in circumstances where direct microscopic examination is liable to error, for example in the examination of the interior of sacks of material in the sampling of which mould mycelium might be so broken up as to be identifiable only with difficulty. A further series of experiments was therefore carried out in which the dried grass, instead of being spread out in flat dishes, was artificially damped and kept in storage in large bottles.

Examination of the nature of the mould growth at different humidities demonstrates a further point of considerable academic interest. It will be seen from Fig. 5 that the period between the formation of mycelium and actual fructification of the mould increases as the humidity is lowered. At 100% relative humidity there is an intervening period of scarcely 24 hr., at 90% the period is 3 days, at 80% a week, at 74% it is 5 weeks, and at 72% as long as 14 weeks. The sample stored at 70% humidity did not show mycelium formation until about the 300th day, and at the present date (520th day of storage) no fructification has yet taken place. At this later humidity the mould spores present in the original grass have therefore been able to lie dormant (presumably becoming acclimatized to the relatively low humidity) for nearly a year, but have subsequently been able *without any detectable alteration in the surrounding conditions* to form a rather sparse mycelium. The mould has since continued to grow vegetatively, but has so far been unable to produce spores.

PLATE COUNTS IN RELATION TO MOULD GROWTH

The striking nature of the above results appeared to justify a more extensive study of the development of mould growth in the dried grass. It was hoped by this means (a) to elucidate the factors responsible for the observed delay in mould growth at the lower humidities, (b) to ascertain whether mould growth could be detected at an earlier stage by plating methods, and (c) if so, to use these methods to study the rate of mould growth at different humidities and/or moisture contents.

The first series of these experiments was carried out by exposing thin layers of dried grass in Petri dishes in desiccators of known humidity, as already described. The desiccators were stored at room temperature. The dishes were examined frequently under a low power objective in order to detect mycelium formation, at which time a sample equivalent to 1 g. of the original dried grass was removed, shaken well with sterile tap water and plated out in several dilutions on dextrose yeastrel agar (pH 6.8) and beerwort agar (pH 3.5). The plates were incubated at 22°C . for 4 days. Periodic examination of the original dishes of dried grass was continued until fructification had become established, when a second sample was plated out. A third sample was plated out after a further interval equivalent to roughly half the period required for the establishment of fructification, at which time it was assumed that

In Fig. 5 the periods taken to reach these two stages have been plotted separately. The resulting curves demonstrate three important points. In the first place it will be seen that the curves themselves are extraordinarily regular: they show clearly that the rate of development of mould growth is a definite function of the relative humidity.¹ Thus mycelium formation was visible within 3 days at 100 % relative humidity, within 5 days at 90 %, 9 days at 80 %, 50 days at 75 %, and over 300 days at 70 % relative humidity. In the second place the delay in mould growth at these latter humidities is itself an extraordinarily interesting and significant finding. It has usually been assumed that if no mould growth occurs within 2-3 weeks of storage, a product

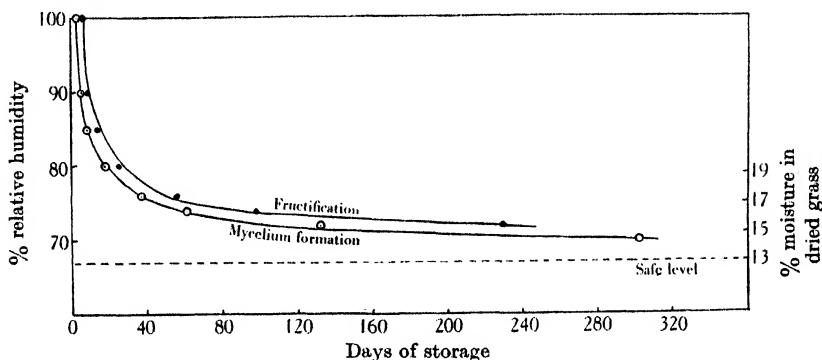


Fig. 5. Rate of mycelium formation and of fructification of moulds on dried grass stored at various relative humidities (room temperature).

can be considered immune from deterioration. The present results show that this assumption is incorrect and that, in order to determine the true degree of safety of storage conditions, very long period trials are essential. In the third place the results (probably because they are based on such exceptionally long period trials) fail to confirm the widely held view that relative humidities below 75 % effectively prevent mould growth: they show, in fact, that mould growth may take place at humidities as low as 70 %, and that for reasonably safe storage a level of 67 % should not be exceeded. This corresponds to the maximum moisture content in the dried grass of roughly 13 %. It will be shown later that even this low value does not confer absolute immunity from mould growth.

¹ It may be noted that later experiments with a wide variety of feeding stuffs indicates clearly that the relative humidity rather than the moisture content of the material is the primary factor affecting mould growth.



(a) 35 days' storage at 77 % relative humidity: sparse mycelium.



(b) 35 days' storage at 90 % relative humidity: general fructification of a variety of mould types.



(c) 520 days' storage at 70 % relative humidity: generalized network of mycelium.



(d) 520 days' storage at 72 % relative humidity: sparse fructification of a single mould type.

Plate 1. Microphotographs illustrating differences in mould growth at various relative humidities ($\times 15$).

typical of local practice on light soils, was varied in 1939 to include grubbing both 3 and 6 in. deep.

For the surface cultivations of the control plots, wide flat sweeps on the motor-hoe had proved very satisfactory in 1938, and these were used for all of the control plots in 1939. It had been found that although irregularities of the soil surface caused a very slight occasional mulching effect on the first operation, this became less with successive scrapings. The sweeps were arranged to move any soil loosened by scraping towards the plants. The blades were sharpened in order to minimize soil disturbance, thus providing a more effective comparison with the ridging and grubbing treatments.

A possible effect of fertilizer placing on the extent of root-pruning damage was investigated by splitting the plots for two methods of fertilizer application. It was considered that a concentration of fertilizer near to those roots which are out of reach of the grubbing implements might be an advantage, and the plots were therefore split to compare the sowing of fertilizer in the planting furrows with broadcasting followed by harrowing in on the flat.

The striking effects observed in 1938 of the competition of small weeds with the potato crop were considered worthy of further investigation in 1939. An attempt was made to extend this study by direct sampling for an estimate of weed density.

The trials for 1938 had shown only a suggestion of the expected advantage of early, as compared with later, grubblings. This comparison was therefore dropped in favour of a study of depth of working. The frequent grubblings were begun as soon as the plant rows were clearly visible, the necessity of avoiding accidental pre-emergence damage being doubly important under the conditions of an experiment.

The standard error for the total yield data in the two previous years had been rather high (12.5 and 12.9 % respectively), although such variation is not unusual in plot trials. The rather small size of the plots (approximately $\frac{1}{100}$ acre in both years) was a possible contributory factor, and in 1939 the plot area was increased to $\frac{1}{70}$ acre.

Details of the experimental design.

Four randomized blocks of eight plots each were used. This gave four replications of the seven treatments and of one dummy comparison.

Details of treatments.

F 6. Five cultivations to a depth of 6 in., the last being followed immediately by bouting-up.

F 3. Five cultivations to a depth of 3 in., followed immediately by bouting-up.

Nc 6. Two 6-in. cultivations only. Plots kept weed-free by additional surface scraping. Bouted-up after second cultivation.

Nc 3. Two 3-in. cultivations only. Otherwise as for Nc 6.

Nw 6. Two 6-in. cultivations only, but with no additional weed control. Bouted-up immediately after the second cultivation.

Nw 3. Two 3-in. cultivations only. Otherwise as for Nw 6.

S. No soil-stirring cultivations, but plots were kept weed-free by scraping with flat hoes, working at about $\frac{1}{2}$ -in.

S. Ditto (dummy comparison).

Fertilizer application.

All plots were halved for the application of fertilizer by two methods. The half-plots were harvested separately.

H. Fertilizer harrowed-in on the flat before drawing out the furrows for planting.

B. Fertilizer applied in the furrows during planting.

Size of plots.

The harvested area of each plot consisted of four 20 yd. rows 32 in. apart. All plots were separated by guard rows, and outer plots were protected by double guard rows. The harvested area of each plot was thus approximately $\frac{1}{70}$ acre.

Planting details.

Variety: Majestic (certified Ross-shire seed).

Spacing: Rows were 32 in. apart, and sets were 15 in. apart.

Bouting-up.

This was done by a double mould-board hand plough, drawn by a tractor on pneumatic tyres.

The equipment was taken throughout the crop, the plough being raised to slide along the surface when traversing S plots.

Some haulm-crushing by the tractor tyres occurred, but this was shared equally by all plots, and the following weeks of intermittent rain brought rapid recovery, removing most traces of the damage. A little of the haulm on the N and F plots remained buried.

Site of the experimental plots.

The much larger area of the 1939 experiment was arranged to cover most of the combined sites of the two previous experiments. A con-

trasting soil type would have been an advantage, but no suitable alternative site was available. As in previous years, the blocks were arranged at right angles to the direction of slope of the water-table, and the long narrow plots were paralleled to the slope. Thus any effect on yield of differing moisture supply from the water-table was eliminated in the statistical analysis of the results. The results of this experiment are given in Table 4.

Table 4. *Total yields and percentage ware for 1939 experiment*

	(1) Treatment means							
Yield (tons/acre)	S 11.78	F 6 10.51	F 3 10.98	Nc 6 11.05	Nc 3 10.93	Nw 6 8.93	Nw 3 10.74	s.e. 0.70
% ware	90.6	90.7	90.7	91.1	90.0	89.1	90.2	0.49
Nc 6–Nw 6. Yield difference 2.12 ± 1.00 ; % ware difference $\pm 2.0 \pm 0.69$								
	(2) Effect of frequency of cultivation							
	S No cultivations	F Five cultivations	Nc Two cultivations (weed-free)	Nw Two cultivations (weedy)				
Yield	11.78	10.75	10.99	9.67				± 0.50
% ware	90.6	90.7	90.5	89.7				± 0.35
	(3) Effect of depth of cultivation (weed-free plots only)							
	$\frac{1}{2}$ in. surface scrapping	3 in. cultivations	6 in. cultivations					s.e.
Yield	11.78	10.88	10.78					± 0.50
% ware	90.64	90.3	90.9					± 0.35

Where weeds were removed, there were again no signs of any response by the potato crop, either to ridging-up with two preliminary grubblings, or to ridging with five preliminary grubblings as compared with surface scraping. Of the four comparisons between the F and S treatments provided by the four blocks, three were in favour of S. The small difference in the mean yields, below statistical significance, even suggested some reduction in yield from the ridging-up and a slight further reduction from additional grubblings. The percentage ware was very uniform. There is a similar suggestion of a slight further decrease in yield for the 6 in., as compared with the 3 in. grubbing. The percentage ware shows, if anything, a trace of the opposite effect. These differences are not, however, established and must be considered to be due to the various factors of experimental error.

Weed competition was less intense in 1939, since the area had been partially cleaned by the preceding potato crops. The weedy plots gave, however, a significantly lower mean yield than the remaining weed-free plots, though almost the whole lowering was due to the Nw 6 plots, i.e. those cultivated to 6 in. The probable reason was that the two grubblings

on the Nw 3 plots were done on 19 June and 1 July, while on the Nw 6 plots the first grubbing was delayed until 25 June. The difference of yield of 2.12 tons per acre was produced by comparatively small weeds which nowhere dominated the haulm. The weeds were destroyed twice during the growing season and were largely smothered by the haulm after the second cleaning. There was a severe drought in June, and this result probably illustrates the effect of weed cleaning at a critical stage of the competition between the crop and the weeds for their water supply.

AN ESTIMATE OF WEED DENSITY

On 18 June, just before the first grubbing of the Nw 3 plots, an estimate was made of the weight of weeds on each of the Nw plots. A light wooden frame was constructed to enclose an area of $\frac{1}{2}$ m. sq. This was placed centrally in the space between the rows, and the weeds within the frame were pulled. The roots were cut off to ground level by scissors, and the plants were immediately weighed on a spring balance. The whole operation was thus carried out fairly rapidly in dry sunny weather, the operator wearing tennis shoes in order to minimize trampling of the soil. This trampling on the dry soil had a negligible effect. One random sample was taken in this manner in each of the five inter-row spaces of each subplot. The sample means thus obtained were observed to agree closely with an eye estimate of the weediness of the plots.

Fig. 1 shows the potato yields in lb. per half plot plotted against the weed weight on 18 June for both the Nw 3 and Nw 6 series. There is a high positive correlation between the weed density and plot yield instead of a negative correlation as would be expected. The more fertile plots grew both more weeds and more potatoes than the less fertile.

Two pairs of (N) plots in each block differed only in the presence or absence of weeds. Their differences in yield are set out below, with the corresponding weed density estimates. A negative sign indicates an apparent increase of yield due to weeds.

Block	Yield difference in lb.		Yield difference in lb.	
	Nc 3-Nw 3	Weed density in gm./sq. m.	Nc 6-Nw 6	Weed density in gm./sq. m.
I	- 2	184	+ 96	220
II	- 29	400	+ 79	488
III	- 10	844	+ 96	600
IV	+ 46	404	+ 4	1188
Mean yield per plot in lb.	176		162	

The correlation ($r=0.263$) is insignificant for eight pairs of values. The shallow-grubbed plots, grubbed in mid-June, show no average reduction in yield from the weed competition. The deep-grubbed plots, grubbed a week later, show considerable yield reductions, but these are

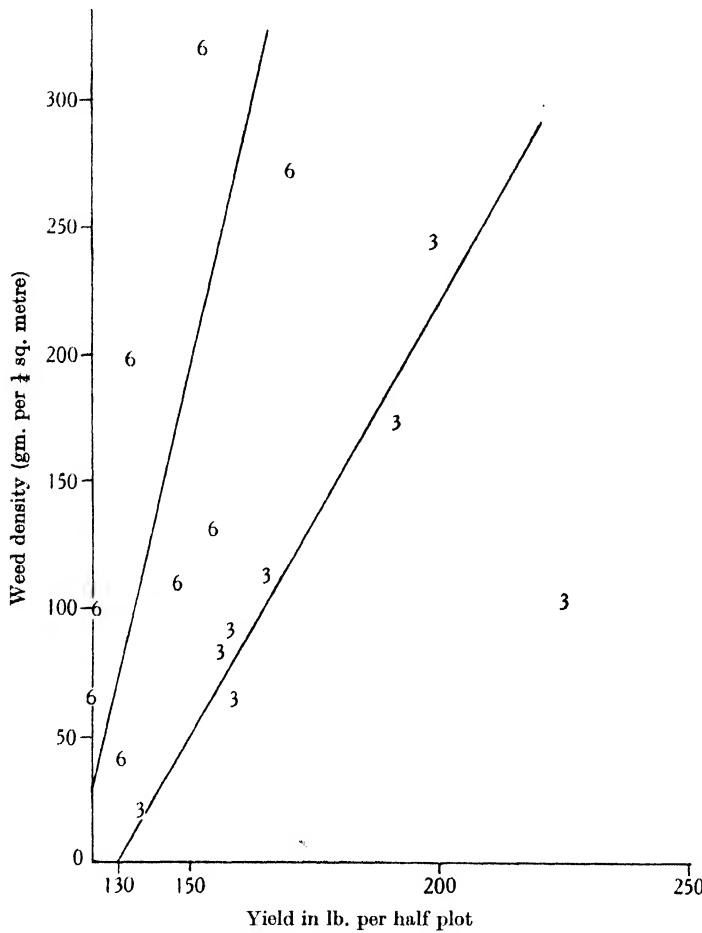


Fig. 1. Weed density and crop yield.

6 refers to Nw 6 plots; 3 refers to Nw 3 plots

not significantly related to the weed density as shown by the sample. This probably indicates that the method of sampling the fresh weight of weeds does not give any satisfactory measure of the intensity of their competition with the crop.

THE EFFECTS OF FERTILIZER PLACING

The effect of placing the fertilizer in the bouts before planting as compared with harrowing it in before drawing out the bouts was small. There was a small apparent gain of 2.5% in yield due to placement which was far below statistical significance.

The fertilizer was, however, subjected to heavy rain for a week while lying in the furrows, on a very well-drained sandy soil. This would be expected to produce a decrease in yield as compared with the half-plots on which the fertilizer had been protected from rain by harrowing in. The result, however, was that six out of the eight treatments, and the experiment as a whole, showed slight suggestions of a gain. This suggests that there may be a real advantage in this method of placing the fertilizer as near as possible to the sets, although the experiment does not produce any reliable evidence for this.

The fertilizer placing had no effect on the size of the tubers. The very small differences in the means shown by percentage ware are all well within the margin of experimental error.

The fertilizer placement did not appear to give any marked effect on any of the treatments, though there was a suggestion, well below statistical significance, that the effect of weed competition was less noticeable on the yield when the fertilizer was placed closer to the plants. As a corollary, the weight of weeds harvested on 18 June was smaller on the half-plots in which the fertilizer was placed than on those in which it was harrowed in, but again the difference of weed weight was well below statistical significance.

The placement of the fertilizer had, however, a definite effect on the percentage of the ware potatoes in the scraped series S spoilt by greening. Spoilt ware on the half-plots in which the fertilizer was placed amounted to 2.5% as against 4.5% on the plots in which it was harrowed in, and this 2% reduction was statistically significant.

**THE EFFECT OF A SURFACE MULCH ON THE
MOISTURE CONTENT OF THE SOIL**

In the 1937 experiment, eighteen plots, forming half of the Latin square, were given the same cultivation treatments as the potato plots, but were kept bare and clean of weeds by applying a light dressing of arsenious weed killer. The moisture content of these plots was determined three times, on 18 July, 2 and 28 August, during a spell of dry

weather, the July and August rainfalls being only 1.28 and 1.13 in. The moisture content of each plot was determined on three bulked 2 ft. samples which were thoroughly mixed, quartered and dried at 110° C. for 24 hr. The difference in moisture content between the frequently mulched (F series) and the unmulched (U series) plots was 0.03–0.54 and –0.28 % on the three sampling dates respectively giving a mean loss of water due to mulching of –0.26 %, which is well within experimental error.

These determinations were repeated in 1938. The spring and summer were hot and dry, the June and July rainfalls being 0.75 and 1.17 in. Samples were taken on 16 and 29 June and 17 July midway between the rows of the plants down to a depth of 18 in., and were dried at 110° C. for 24 hr. as before. The difference in moisture content between the frequently mulched (F and F' series) and unmulched (U and U' series) plots was 0.79, 0.32 and –0.51 % on the three sampling dates respectively, giving an apparent mean gain of moisture content of 0.22 % due to mulching. All the differences, however, were just within experimental error.

Only one set of moisture determinations was made in 1939 on 5 July after a long dry spell in June, and after this the weather broke. The methods used were similar to those used in 1938, except that bouting had already been done on 1 July, so that the depth of sampling was 21 in. in the bouts, 15 in. in the furrow and 18 in. on the flat. The plots (F 3) on which a 3 in. mulch was maintained continuously had a moisture content 0.13 % less than the unmulched (S) plots. This was well within experimental error.

The experiments on the effect of a surface mulch, in the absence of weeds, on the mean moisture content of the first 18 in. or 24 in. of the soil showed that it gave a mean increase of moisture content of 0.22 % in 1938 and a mean decrease of 0.26 and 0.13 % in 1937 and 1939 over the unmulched soil, giving a mean reduction of 0.06 % for the 3 years. The mulch thus had absolutely no effect on the moisture content of the first 18 in. of soil.

Mulching may, however, cause a definite loss of water as compared with clean weeded land if the water-table is near the surface. In 1938 one block, block VI, had a water-table at a mean depth of just less than 3 ft. 6 in., while the mean depth on the other five blocks varied from about 4 ft. 9 in. to 9 ft. 6 in. The high water-table kept the plots of this block wetter than the other blocks, and mulching caused a pronounced lowering of moisture content, instead of the gain which the traditional

capillary hypothesis would predict. The results of this sampling, which was done on 17 July, were:

	Depth of water-table	Moisture content		Mulch-unmulched
		Mulched (F')	Unmulched (U')	
Block VI	About 3 ft. 6 in.	9.80	13.16	-3.36
Blocks I-V	Below 4 ft. 6 in.	8.09	7.99	+0.10

This may only have been a chance effect, for it was not shown at all clearly in 1937. Three separate samplings were taken on land carrying no crop, and the mean results were:

	Approximate mean depth of water-table	Moisture content		Mulched-unmulched
		Mulched (F)	Unmulched (U)	
Block VI	About 3 ft. 3 in.	13.60	14.92	-1.32
Block V	About 3 ft. 6 in.	13.64	13.16	0.48
Blocks I-IV	Below 4 ft.	11.09	11.28	-0.19

The results of the three separate samplings were perfectly concordant, the difference mulched-unmulched being:

Sampling date	18. vii. 37	2. viii. 37	28. viii. 37
Block VI	-1.28	-1.99	-0.70
Block V	0.50	0.43	0.51
Blocks I-IV	0.24	-0.43	-0.37

A possible explanation of this difference is that in both 1937 and 1938 the two plots concerned in block VI were contiguous, while they were not in block V, and as five water-table heights were taken just outside the experimental area, it is possible the apparent anomaly of block V is due to the water-table being higher under the mulched plot than under the unmulched.

Mulching does, however, conserve moisture if the comparison is made between clean mulched land and weedy land. In 1938 the difference in moisture content on block V between the weed-free plots and the plots of series N, which only received two grubblings and no hand-weeding, was a loss of 1.44 and 1.33 % of moisture content due to weeds on 16 and 29 June respectively, which lie well outside the limits of experimental error. The yield, as already noted on p. 218, was 43 % lower on this weedy plot than on the rest of the block, and it is possible that this great reduction was due to the competition of weeds with the potato plants for the strictly limited supply of water. Further evidence can be found for this explanation since the yield of potatoes per block seemed to be correlated with the mean moisture content of each block on 18 July in 1937 and on 17 July in 1938, as is shown in Fig. 2. It is

interesting to note that the block which fell off the regression line in 1938 was very close to the area occupied by the block which fell off in 1937.

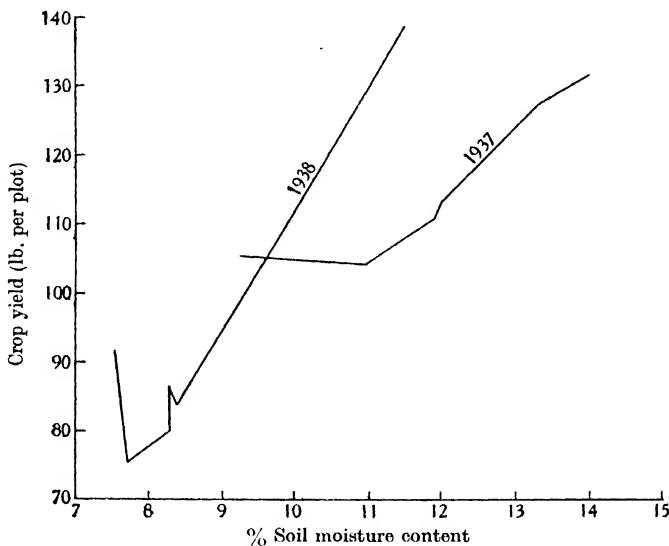


Fig. 2. The relation between crop yield and the moisture content of the first 18 in. of soil. (Block means.)

THE EFFECT OF THE HEIGHT OF THE GROUND-WATER-TABLE ON THE MOISTURE CONTENT OF THE SOIL

The height of the water-table was observed in several plots in both 1937 and 1938, and it was found to vary between about 3 and 10 ft. In both years moisture contents were available, in 1937 there were three separate samplings on the bare plots of the first 2 ft. of soil and in 1938 only one of the first 18 in. of soil, but since it was taken from the bottom of the furrow between two ridges, it really extended from a depth of 2 ft. up to a depth of 6 in. The moisture contents of these samples were:

Year	Water-table	
	Between 3-4 ft.	Below 4 ft.
1937	14.0	11.2
1938	11.5	8.0

DISCUSSION OF RESULTS

The yield of potatoes in these trials was definitely above the annually published national average for main crop potatoes in two of the years and just above in the third, although only very moderate dressings of dung and fertilizers were used. Hence a high standard of yield was obtained on light land without deep or frequent soil stirring.

The potato crop proved unexpectedly insensitive to inter-row tillage. It was, in fact, surprisingly so in 1938, when one set of intensive grubblings which was given a fortnight later than usual, caused no significant reduction of yield although it must inevitably have caused more extensive root pruning at a stage when root spreading had reached the centre of the row. Throughout the trials the crop seemed to be remarkably adaptable and to grow well under a wide variety of cultural practices.

The susceptibility of the potato yield to competing weeds was in marked contrast to its lack of sensitivity to cultivation treatments. It is possible that this susceptibility may have been due to some specific limiting factor, for which both crop and weeds compete, and if so this factor was probably shortage of water rather than shortage of any particular nutrient.

This demonstration of the negative result of cultivation to produce significant increases of yield would not satisfy the practical grower if these cultivations are the only way of keeping the weed population low or if they reduce the time spent on spraying and harvesting or if they benefit the succeeding crops in the rotation.

These results suggest that shallow sweeps or light spring-tine weeders could replace grubblings and bouting on light soils with considerable saving of time and fuel, if they were used fairly frequently when the crop was still young. A shallow cultivation at about 1 to 1½ in. appears to be adequate for weed destruction on such soils.

Inter-row cultivations are often the easiest way to obtain a sufficiency of fine tilth for bouting-up, and so long as bouting-up is an essential part of potato culture, many of these tillages will probably be done for this reason alone. Bouting-up is probably done for three reasons. First it reduces the damage the sprayer does to the haulms when it goes through the crop. Fitting efficient haulm lifting guards to the tractor and sprayer wheels, such as are already used with success in laid corn, would probably prove a more efficient way of reducing haulm damage than bouting-up. Secondly, the bouts reduce greening of the tubers, but very low ridges are all that are needed for this purpose. Thirdly, the existing potato harvesting

machinery works much better on ridged than on flat land. But this limitation can be overcome, on light land at any rate. The potato-lifting plough, fitted with a double set of lifting fingers, was used successfully for harvesting unridged guard-rows at Ottershaw Park, and this implement is much used in South Lincolnshire. The spinner undoubtedly works more easily with fairly high ridges, especially on heavy soils. The power-operated chain-elevator digger, a potato harvesting implement used almost exclusively in some potato-growing areas of the U.S.A., appears particularly suited to modern tractor conditions. It is little known in England, but Hardenburg (1934), in observations on 254 fields of potatoes near New York, reports it to be capable of lifting crops ridged 2 in. high with only a little more damage than when the ridges were much higher. These results suggest that in light soils the grubbing and ridging policy should aim at the minimum height of ridge to which the harvesting equipment can be adapted.

The effect of intensive cultivation of the potato crop on succeeding crops depends on the weed population. If the potatoes are grown as the 'cleaning crop' in a rotation, then certain perennial weeds may best be destroyed by frequent deep grubbing. Again, however, it is important that the grubblings on light soils be given with the definite object of weed destruction and that unproductive work in excess of these requirements should be avoided.

These experimental results may not be applicable to heavy soils. In the first place a loose mulch on heavy soils may prevent the soil surface from baking into hard clods and thus facilitate the destruction of the weeds. It may not be possible to obtain sufficient fine soil to protect the tubers without cultivating the soil 2-3 in. deep. Harvesting requirements may also make higher ridges desirable.

Soil moisture content has been shown by modern studies to be a highly variable quantity, and a more extensive programme of sampling would have been desirable, if minor effects of the 3 in. mulch on the moisture of the first 2 ft. of soil were to be reliably detected. The samplings were, however, sufficiently intensive to detect any moisture-conservation effect of the mulch great enough to be of economically important benefit to the crop. No such effect occurred in three seasons of widely varying rainfall. The range of water-table depths was such that capillary rise of soil moisture was important for some complete replications, and unimportant for others. Thus the experiments included conditions representative of the principal range of water-table situations likely to occur on light arable soils. For none of these conditions was the mulch effective.

An explanation of this ineffectiveness of the mulch to conserve moisture is offered by the relation observed between the moisture content of the upper soil and the depth of the water-table. The results of research into the movement of water in soils show that important capillary movement of soil moisture is to be expected only where a continuous supply from a water-table is occurring, and then only through a strictly limited distance. This distance, on the sandy soil studied, proved to be no more than 4 ft. Where the water-table lay deeper, any water lost by evaporation from the upper soil could not be replaced from below. No capillary rise therefore existed, thus rendering the mulch ineffective. The lack of effect where capillary rise did exist, may possibly be explained by the rapid replacement from below of any water lost from the unmulched plots, so that on these plots the layer dried by evaporation was no deeper than that of the comparable 3 in. soil mulch. The demonstration of the limit of capillary rise in a natural soil under field conditions is rarely possible, and the result is, therefore, of considerable interest.

SUMMARY

The experiments conducted at Ottershaw Park in the years 1937, 1938 and 1939 indicate that for a well-drained sandy loam, under a considerable range of moisture-supply conditions, main-crop potatoes do not respond in the absence of weeds to ridging-up, or to deep or frequent inter-row grubblings, by any increase in yield or in the percentage of ware. The crop showed successful powers of adaptation to a range of contrasting inter-row tillage treatments.

The potato crop showed considerable sensitivity to weed competition in the early stages of growth. This indicates that it is of great importance to maintain the crop in a weed-free condition during this early period.

Inter-row tillage operations on this type of soil should, therefore, be designed to destroy weeds and to provide moderate cover for the tubers. Intensification of such tillage beyond these limits is not of direct benefit to the plants.

No moisture-conservation effect of any importance was produced by a 3 in. soil mulch during dry weather, even when the water-table lay within 4 ft. from the surface.

A significant upward movement of water from the water-table to the surface soil was observed, but this was limited to a vertical distance of approximately 4 ft. for the soil type described. Beyond this limit the surface soil was apparently unaffected by the depth of the water-table.

These results may not be applicable to heavier soil types. It is probable, however, that they would hold good for most sandy loams.

The results indicate the desirability of carrying out further experiments on contrasting British soil types, especially in the important potato-growing areas.

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THE NUTRITION OF THE BACON PIG

VI. THE MINIMUM LEVEL OF PROTEIN INTAKE CONSISTENT WITH QUICK GROWTH AND SATISFACTORY CARCASS QUALITY (PART III)

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INTRODUCTION

EARLIER investigations in this series showed that a diet supplying only half the amounts of protein supplement hitherto considered necessary for optimum growth of bacon pigs gave as good results, from the stand-points of rate of growth, efficiency of food conversion and carcass leanness and quality, as a diet containing the full standard amounts. In the first of these trials (Woodman *et al.* 1939) the protein supplement was composed of a mixture of feeding blood meal, dried separated milk and ex. soya bean meal, while in the second trial (Woodman & Evans, 1940) it consisted of a single protein food, white fish meal, which, in the low-protein treatment, was fed at the rate of 5% of the ration up to 90 lb. and 4% from 90 to 150 lb. live weight. With the object of eliminating the risk of fishy taint in the bacon, fish meal was replaced by ex. soya bean meal, at the 3% level, from 150 lb. to slaughter at about 200 lb. live weight.

THE PRESENT TRIAL (JAN.-JUNE 1938)

In the two trials referred to above, it is probable that the lowest level of protein intake consistent with quick growth and satisfactory carcass quality had not been attained, except perhaps in the earlier stages, when 5% of white fish meal (or its equivalent) appeared to be too low for maximum rate of growth over this part of the feeding period. An effort was made in the present trial to reach a subminimal level by a very drastic cutting down of the protein supplement.

The scheme of the feeding treatments is shown in Table 1. The pigs on the control treatment A received the standard allowances of protein-rich food, consisting of 10% of white fish meal from weaning to 150 lb.

live weight and 5% of ex. soya bean meal from 150 lb. to slaughter at 200 lb. The pigs on treatment B were given only 5% of white fish meal up to 90 lb. and 2% from 90 to 150 lb. live weight, while protein-rich

Table 1. *Scheme of feeding treatments*

	Treatment A (parts by weight)	Treatment B (parts by weight)	Treatment C (parts by weight)
Up to 90 lb. l.w.:			
Barley meal	57	62	65
Weatings	31	31	31
Lucerne meal	2	2	2
White fish meal	10	5	2
Minerals*	—	1	1.6
90-150 lb. l.w.:			
Barley meal	65	73	75
Weatings	23	23	23
Lucerne meal	2	2	2
White fish meal	10	2	—
Minerals*	—	1.6	2
150 lb. l.w. to slaughter:			
Barley meal	80	85	85
Weatings	13	13	13
Lucerne meal	2	2	2
Ex. soya bean meal	5	—	—
Minerals*	2	2	2

* The minerals consisted of 1 part by weight of common salt to 3 parts of ground chalk.

Table 2. *Average composition of feeding stuffs*

	Barley meal %	Weatings %	Lucerne meal %	Ex. soya bean meal %	White fish meal %
Moisture	13.70	14.10	9.60	13.10	11.50
Crude protein	11.97	16.12	24.37	44.13	63.17
Ether extract	1.87	3.93	3.65	0.44	3.30
N-free extractives	64.89	56.50	35.79	32.92	0.99
Crude fibre	4.87	5.89	19.22	4.26	—
Ash	2.70	3.46	7.37	5.15	21.04

Table 3. *Feeding chart**

l.w. in lb.	lb. meal	l.w. in lb.	lb. meal
20	1.10	120	5.30
40	2.10	140	5.90
60	3.00	160	6.45
80	4.00	180	6.70
100	4.60	200	7.00

* Change in meal allowance shown for live-weight increments of 20 lb.; adjustments should be made for intermediate live weights, so that the meal supply may be altered week by week in accordance with the weekly weighings of the pigs. For the case of the individually fed pigs, it was possible to make the changes of ration as each pig arrived at 90 and 150 lb. live weight. With the group-fed pigs, however, the change-over at 90 lb. was made when the group average had reached this live weight. As each group-fed pig reached 150 lb., its allowance of food was weighed out from the finishing mixture and incorporated in the total meal allowance for the group. This ensured the desirable reduction of the amount of fish meal in the diet well before the heaviest pigs in the group arrived at slaughter weight.

food was omitted altogether from 150 lb. to slaughter. The reduction was carried still further in the case of the pigs on treatment C, as little as 2% of white fish meal being fed from weaning to 90 lb., while from this stage to slaughter at about 200 lb. live weight, no protein-rich food at all was included in the diet.

PRE-SLAUGHTER RESULTS

The weaners were brought straight from the sows into the experimental piggery on 28 December 1937. They all belonged to the Large White breed. Eight of the litters came from the University Farm herd and had been out on grass with the tethered sows during the suckling period, during which period they had access to a weaner's mixture containing 10% of white fish meal. The two remaining litters (Mis. and Bar.) had been bought from neighbouring farms. Both these litters were sty-reared and had received green food in the form of cabbages, but whereas litter Bar. had been given food containing 8% of white fish meal, litter Mis. had received mainly weatings with no fish meal.

All the piglets were kept on ration A, containing 10% of white fish meal, during the preliminary period of settling down to the new conditions. The distribution of the pigs in the experiment was made on the basis of their live weights on 3 January. They were brought on to the experimental rations on 5 January and were weighed again on the mornings of 9, 10 and 11 January, the average of these three weighings being taken as the initial live weights in the trial. The comparison of the effects of the three feeding treatments was continued until 25 April (15 weeks), when the first consignment of pigs was sent to the bacon factory, but as in previous trials, complete records were kept until all the pigs had reached bacon weight at about 200 lb. For fuller details of the technique of the pre-slaughter work, the reader should consult the first publication of this series (Woodman *et al.* 1936).

A summary of the data for live-weight increase and meal consumption over the 15 weeks of comparison is given in Table 4, which also shows how the pigs were distributed in both the individual-feeding and group-feeding trials.

Comments on Table 4

The means of the data for the pigs over the 15 weeks of the comparison are shown in Table 5.

Table 4. *Live-weight gains and meal consumption over experimental period of 15 weeks (10 January to 25 April)*

Treatment	No. and sex of pig	Individually fed pigs					Group-fed pigs*				
		L.W. on†	L.W. on	L.W. on	Total meal con-	No. and sex of litter	L.W. on†	L.W. on	L.W. on		
		3 Jan.	10 Jan.	25 Apr.	sumed		3 Jan.	10 Jan.	25 Apr.		
Pen I (sow Bar.)											
A	G 32	33	38	175.5	434.80	357	H 2692	40.5	45.5	142.0	
B	G 31	32.5	37	164.0	419.80	1413	H 2673	32	36.5	160.0	
C	G 35	33	37.5	153.5	396.10	1126	H 2701	42	50	184.0	
C	H 38	40.5	44.5	168.0	445.55	1408	H 2715	52	59	198.5	
B	H 29	36.5	40.5	174.0	446.05	820	G 2689	37.5	43.5	194.5	
A	H 36	37	43	192.5	483.00	Bar.	G 33	28	30.5	123.5	
							832	H 2726	30	32.5	150.0
Pen II (sow 1622)											
B	G 2709	28.5	32	142.0	369.05	357	G 2695	36.5	40.5	173.5	
C	G 2707	30.5	33	134.0	356.00	1408	G 2712	47.5	54	206.5	
A	G 2703	29	34	156.5	396.40		Group II (treatment B)				
C	H 2708	33.5	36.5	141.5	373.80	357	H 2694	38	43	146.5	
A	H 2706	31	35	147.0	389.85	1413	H 2677	41.5	45.5	169.5	
B	H 2705	30	33.5	134.0	357.20	1126	H 2697	49.5	57.5	200.5	
Pen III (sow 1413)											
C	G 2875	35.5	39.5	157.5	414.40	1408	H 2716	47	53	187.5	
A	G 2671	35.5	39.5	167.0	444.05	820	G 2688	30	32.5	130.5	
B	G 2672	32	36	160.5	410.55	Bar.	G 34	37.5	41	152.0	
B	H 2683	31.5	35.5	155.0	402.50	832	H 2719	29	33.5	137.0	
C	H 2678	34	37	148.0	395.50	820	G 2687	40.5	41.5	128.5	
A	H 2674	35	40	164.0	439.95	1413	H 2669	30.5	33	135.0	
							G 2681	39	45	184.0	
Pen IV (sow Mis.)											
C	G 46	34	35	131.0	361.95	357	H 2696	34.5	39.5	153.0	
A	G 44	34	37.5	162.0	425.40	1413	H 2684	45.5	51	185.0	
B	G 43	32	35.5	155.0	395.15	1126	H 2698	39.5	44.5	135.0	
A	H 40	35.5	37	168.0	436.00	1408	H 2714	51	58	197.5	
B	H 42	33	34	149.5	388.35	820	G 2686	34.5	39	144.0	
C	H 45	35.5	36	144.5	375.40	Bar.	G 37	43.5	47	125.5	
						832	G 2722	34	36.5	135.0	
Pen V (sow 117)											
B	G 2665	42.5	46.5	178.5	473.20	1413	H 2685	34.5	38	137.5	
A	G 2661	34	37.5	175.0	444.85	Mis.	H 41	32.5	34	111.5	
C	G 2667	34.5	38.5	157.5	414.75						
B	H 2662	43.5	48	184.0	486.15						
C	H 2670	35	39.5	154.5	407.05						
A	H 2668	39	44	172.0	464.80						

* It was not possible, with the pigs available for the investigation, to secure a perfect distribution for the group-feeding experiment, a circumstance that detracts somewhat from the value of the results from this section of the trial:

Total meal consumed from 10 January to 25 April

	Individually fed pigs lb.	Group-fed pigs lb.
By 10 A pigs	4359.1	4506.9
By 10 B pigs	4148.0	4237.5
By 10 C pigs	3940.5	4149.3

† Date of distribution; the pigs were brought on to the experimental diets on 5 January.

Table 5. Mean results over the 15 weeks, 10 January to 25 April

Individually fed pigs								Group-fed pigs							
Treatment	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
	L.W. on 10 Jan.	L.W. on 25 April	total L.W.I.	lb. meal per lb. L.W.I.	L.W. on 10 Jan.	L.W. on 25 April	total L.W.I.	lb. meal per lb. L.W.I.							
A	38.55	167.95	129.40	3.38	43.30	170.55	127.25	3.54							
B	37.85	159.65	121.80	3.41	42.55	157.10	114.55	3.70							
C	37.70	149.00	111.30	3.54	42.75	146.30	103.55	4.01							
S.E.	—	—	1.94	0.039	—	—	6.35	—							
Treatment effects*	A and B	S.	N.S.	—	—	N.S.	—	—							
	B and C	S.S.	S.	—	—	N.S.	—	—							
	A and C	S.S.S.	S.	—	—	S.	—	—							

* S. significant at 5% point, S.S. at 1% point and S.S.S. at 0.1% point.

It will be noted from Table 5 that, at the end of the first 15 weeks of the trial, the differences in feeding treatment had been responsible for significant differences among the individually fed pigs in respect both of live-weight increase and efficiency of food conversion. Over this period, the A pigs on the standard diet containing 10% of white fish meal made significantly higher live-weight gains than the B pigs (5% of white fish meal up to 90 lb. and 2% from 90 to 150 lb.) and the C pigs (2% of white fish meal up to 90 lb. and none thereafter). It will further be observed that the B pigs averaged a significantly higher live-weight increase and better efficiency of food conversion than the C pigs over this period of the trial.

Table 6. *Analysis of results for individually fed pigs (means of results for 10 pigs in each treatment)*

Similar differences are to be noted in the results from the group-feeding trial. The significance of the differences between the efficiencies of food conversion could not be computed in this case, since the separate meal consumption of the animals in the groups was not known. That the group-feeding technique was much less sensitive than that of the individual-feeding experiment, however, is apparent from the statistical analysis of the live-weight gains, since although the difference between the means of the live-weight gains in the A and B groups was fairly substantial, it was not possible to show that this difference was significant.

The results for the individually fed pigs are analysed in greater detail in Table 6, the figures in this case including the comparison for the whole period of feeding from weaning to slaughter at 200 lb. live weight.

Comments on Table 6

Significant differences attributable to the differences in the protein content of the three feeding treatments were confined almost entirely to the earliest stage of the feeding period, namely, from weaning to 90 lb. live weight. The advantage during this period lay very clearly with the pigs receiving 10% of white fish meal (treatment A), whilst even the B pigs, receiving 5% of fish meal, made significantly better progress than the pigs on treatment C, which received only 2% of fish meal in their food. This behaviour confirms a previous conclusion from this series of investigations that the inclusion in the diet of only 5% of white fish meal does not enable young pigs to display their fullest capacity for growth during the period immediately following weaning.

The results for the second period from 90 to 150 lb. live weight, however, are in striking contrast to those of the first period. The A pigs during this second period were still receiving 10% of fish meal, while the allowance of the B pigs had been reduced to 2% and that of the C pigs eliminated altogether. Despite these differences in feeding, no significant differences were found between the treatment means for the number of days required for the pigs to grow from 90 to 150 lb. live weight, the daily rate of live-weight increase and the lb. meal required per lb. of live-weight increase.

The results for the final period (150–200 lb. live weight) are even more striking. The A pigs were now receiving 5% of ex. soya bean meal, while the B and C pigs were fed rations containing no such protein-rich food. The mean results for the A and B pigs over this period were almost identical, but the C pigs actually gained on the pigs in both these

treatments. The superior rate of live-weight increase of the C pigs did not quite reach statistical significance, but the difference in their favour in respect of the efficiency of food conversion was significant at the 5% point.

It is not surprising, therefore, that the results for the whole trial from weaning to 200 lb. live weight (see Table 6) do not reveal such marked treatment differences as are discernible when the comparison is restricted to the results for the first 15 weeks of the trial (see Table 5), a period which does not include the results in the final fattening stages. A statistical analysis of the results from weaning to 200 lb. live weight revealed no significant effect of feeding treatment as between the A and B pigs or as between the B and C pigs. It is only when the results for the extreme treatments A and C are compared that significant effects are evident. This is shown by the treatment means for the number of days required and the rate of live-weight increase per day, but not by the food conversion figures.

That the main effect of the different levels of protein supply was restricted to the immediate post-weaning period is further brought out by adjusting the results for all the pigs to a common initial live weight of 60 lb., in which comparison no significant effects of feeding treatment are discernible (see Table 7).

Table 7. *Results for individually fed pigs adjusted to a common initial live weight of 60 lb. (treatment means)*

Treatment	60-200 lb. l.w.		
	Days required	lb. l.w.i. per day	lb. meal per lb. l.w.i.
A	104.2	1.35	3.81
B	105.8	1.33	3.82
C	105.9	1.32	3.80
S.E.	1.41	0.018	0.045
Treatment effect	N.S.	N.S.	N.S.

The group-feeding trial, although lacking the satisfactory degree of accuracy associated with the individual-feeding experiment (see Table 4), broadly confirms the foregoing findings (see Table 8).

It may be concluded that a diet supplying 5% of fish meal up to 90 lb. live weight, 2% from 90 to 150 lb. and no protein-rich food from 150 lb. to slaughter at about 200 lb. live weight will give almost as good results, from the standpoints of mean daily live-weight increase and efficiency of food conversion over the whole period, as one that provides

10% of fish meal from weaning to 150 lb. and 5% of a protein-rich food such as ex. soya bean meal from 150 to 200 lb. live weight. The small differences noted in the present trial were not statistically significant.

Table 8. *Results for group-fed pigs (treatment means)*

Treatment	Weaning to 200 lb. l.w.		60-200 lb. l.w.	
	Days required	lb. l.w.i. per day	Days required	lb. l.w.i. per day
A	126.2	1.26	105.1	1.34
B	133.6	1.19	107.4	1.31
C	139.6	1.14	113.6	1.24
S.E.	5.61	0.037	3.21	0.036
Treatment effect	N.S.	N.S. A>B	N.S.	N.S.
	—	N.S. B>C	—	—
	—	S. A>C	—	—

Further, a diet supplying as little as 2% of fish meal up to 90 lb. live weight and no protein-rich food at all thereafter has been shown to give results that are not very seriously inferior, particularly when viewed against the background of wartime conditions, to those obtained on the standard diet. It is true that the reduction of the fish meal supply to 5% or less retards to a significant extent the rate of growth up to 90 lb. live weight, but this initial setback, according to the results of this and the writers' previous investigations, tends to be wiped out during the later stages of feeding. It may be argued, on the basis of these findings, that if two comparable weaners, X and Y, are so fed that X, in the period immediately following weaning, is caused to grow at the quickest possible rate, while Y is growing at a rate somewhat below the maximum of which it is capable, then, under comparable conditions of feeding during the later stages, Y will grow somewhat more quickly than X and tend to make up for the initial setback.

It would seem, in the case of Y, as if a reserve of growth impulse, unexhausted during the early stages, remains over for coming into play in the later stages.

The point may be raised that the barley used in this investigation (11.97% protein) was richer in protein than average feeding barley (10% protein) and that this would tend to mitigate the effect of the reduced supply of white fish meal. Against this, however, may be set the fact that the experimental diets contained much smaller proportions of weatings (16.1% protein) than are commonly included in pig rations (see Table 1).

POST-SLAUGHTER RESULTS

The reader is referred to the first paper in this series for a detailed explanation of the technique of the post-slaughter work (Woodman *et al.* 1936). Considerations of space make it impossible to record all the measurements of the sixty carcasses, and only the averages for the pigs in the three feeding treatments, together with the standard errors, will be given.

Table 9. *Effect of feeding treatment on thickness of back fat and belly streak (treatment averages)*

Treatment	Back fat*			Belly streak†				
	(a) mm.	(b) mm.	(c) mm.	Mean mm.	(a) mm.	(b) mm.	(c) mm.	Mean mm.
(1) Individually-fed pigs								
A	53.60	26.90	37.25	39.25	28.30	35.75	40.05	34.70
B	52.60	26.40	35.85	38.29	27.20	35.85	40.65	34.56
C	52.85	26.05	36.20	38.36	29.25	36.55	40.70	35.50
S.E.	1.01	0.87	0.81	0.79	1.08	0.88	0.97	0.83
Treatment effect	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
(2) Group-fed pigs								
A	51.70	23.90	31.00	35.53	24.25	32.45	37.35	31.35
B	53.70	26.45	35.35	38.50	24.65	33.40	38.70	32.25
C	51.05	25.50	34.15	36.90	25.85	33.65	37.95	32.48
S.E.	1.31	1.02	1.24	1.02	1.20	1.11	1.03	0.93
Treatment effect	N.S.	N.S.	S. B-A	N.S.	N.S.	N.S.	N.S.	N.S.
	—	—	N.S. B-C	—	—	—	—	—
	—	—	N.S. C-A	—	—	—	—	—

* As measured, on both sides of the carcass, at (a) the thickest part at the shoulder, (b) the thinnest part along the back and (c) opposite the junction of the third and fourth vertebrae from the curve.

† As measured on both sides of the carcass, (a) opposite the curve, (b) opposite the junction of the fourth and fifth vertebrae from the curve and (c) at a distance below (b) equal to the distance from (a) to (b).

Comment on Table 9

There is no evidence that the substantial lowering of the supply of protein-rich food in treatments B and C below that in the standard treatment A has had any significant effect on the back fat and belly streak measurements.

Comments on Table 10

Among the individually fed pigs, the A pigs on the standard-protein treatment gave, on the average, a larger complete rasher than the B and C pigs, the difference between the treatment means being significant at the 5% point. This was not to be ascribed, however, to significant differences in the area of lean in the rashers, although the tendency was

Table 10. *Effect of feeding treatment on size and leanness of typical rasher (treatment averages)*

Treatment	Belly rasher*				Mid-back rasher*				Complete rasher*			
	"Warm" carcass		Area of weight		Total†		Area of area		Total†		Area of area	
	lb.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.
(1) Individually fed pigs												
A	162.4	95.26	29.93	62.33	128.78	39.70	86.42	224.04	69.63	148.75		
B	162.5	89.37	28.51	58.02	118.73	38.76	77.69	208.10	67.27	135.71		
C	161.2	91.71	27.30	61.60	121.12	37.34	81.34	212.83	64.64	142.94		
S.E.	—	1.94	1.27	1.57	2.57	1.13	2.58	3.82	1.91	3.81		
Treatment effect	—	N.S.	N.S.	N.S.	S. A-B	N.S.	S. A-B	S. A-B	N.S.	S. A-B		
	—	—	—	—	S. A-C	—	N.S. A-C	S. A-C	—	N.S. A-C		
	—	—	—	—	N.S. C-B	—	N.S. C-B	N.S. C-B	—	N.S. C-B		
(2) Group-fed pigs												
A	158.0	86.49	27.95	55.65	114.63	40.36	71.83	201.12	68.31	127.48		
B	159.9	89.39	28.42	58.55	120.29	38.35	79.51	209.68	66.77	138.06		
C	157.9	86.94	25.50	58.84	114.24	35.04	76.76	201.18	60.54	135.60		
S.E.	—	2.22	0.97	2.45	3.00	1.33	3.62	4.55	2.03	5.58		
Treatment effect	—	N.S.	N.S.	N.S.	N.S.	S. A-C	N.S.	N.S.	S. A-C	N.S.		
	—	—	—	—	—	N.S. A-B	—	—	S. B-C	—		
	—	—	—	—	—	NS. B-C	—	—	NS A-B	—		

* See first publication of this series for explanation of these terms (Woodman *et al.* 1936).

† Total area minus sum of areas of lean and fat equals area occupied by bone.

for the A pigs to produce slightly more lean. The main cause of the difference in the size of the rasher was the greater production of fat by the A pigs (significant at the 5% point), a finding that is the reverse of what might have been expected from a consideration of the protein content of the three feeding treatments. Differences of a similar nature are discernible in the figures for the mid-back and belly rashers, and it may therefore be concluded that the reduction of the supply of protein in treatments B and C had not been responsible for an increased production of fat. The minor influence of diet in this connexion is further illustrated when the areas of lean and fat for the individually fed pigs are expressed as percentages of the area of the complete rasher (see Table 11).

Table 11. *Fat and lean as percentages of complete rasher (treatment averages)*

Treatment	Individually fed pigs		Group-fed pigs	
	Lean	Fat	Lean	Fat
A	31.14	66.35	34.20	63.14
B	32.51	65.04	32.05	65.63
C	30.34	67.18	30.30	67.19
S.E.	0.96	0.95	1.46	1.52
Treatment effect	N.S.	N.S.	N.S.	N.S.

Table 12. *Effect of feeding treatment on "eye" muscle measurements (treatment averages)*

Treatment	"Eye" muscle in mid-back rasher					Back fat opposite "eye" muscle cm.	
	Space within line of connective tissue			"Eye" muscle			
	Total area sq. cm.	Area of lean sq. cm.	Area of fat sq. cm.	Length em.	Depth em.		
(1) Individually fed pigs							
A	34.47	28.03	6.44	7.22	5.82	3.22	
B	34.70	28.94	5.76	7.45	5.52	2.91	
C	33.14	27.23	5.91	7.41	5.32	3.11	
S.E.	0.89	0.95	0.45	0.16	0.17	0.12	
Treatment effect	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	
(2) Group-fed pigs							
A	34.63	29.21	5.42	7.58	5.51	2.69	
B	33.37	27.98	5.39	7.48	5.39	3.01	
C	31.71	25.78	5.93	7.57	5.01	2.88	
S.E.	1.03	1.23	0.59	0.19	0.19	0.14	
Treatment effect	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	

Table 13. *Influence of feeding treatment on certain post-slaughter measurements (treatment averages)*

Treatment	(1) Farm-fasted l.w. lb.	(2) L.W. at factory	(3) "Warm" carcass percentage	(4) Length cm.	(5) Flares g.	(5) Fillets g.	(5) Kidneys g.	(6) Sides as % of carcass weight	(7) Iodine value of back fat
(1) Individually fed pigs									
A	201.6	196.3	82.76	76.6	2017	496	252	75.90	68.2
B	202.1	197.4	82.34	77.4	2027	471	251	75.73	67.3
C	200.8	195.5	82.48	76.9	2098	466	244	75.67	65.3
S.E.	—	1.50	0.54	0.53	79	13.8	7.0	0.21	0.53
Treatment effect	—	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	S. A > C
	—	—	—	—	—	—	—	—	S. B > C
	—	—	—	—	—	—	—	—	N.S. A > B
(2) Group-fed pigs									
A	200.6	197.6	79.96	78.4	1726	512	267	75.76	67.5
B	201.3	198.6	80.49	77.0	1757	473	237	75.96	65.5
C	200.8	195.2	80.90	77.6	1848	474	238	74.97	65.8
S.E.	—	1.46	0.46	0.38	109	23.2	7.8	0.36	0.83
Treatment effect	—	N.S.	N.S.	S. A > B	N.S.	N.S.	S. A > B	N.S.	N.S.
	—	—	—	N.S. A > C	—	—	S. A > C	—	—
	—	—	—	N.S. B < C	—	—	N.S. B < C	—	—

(1) After 24 hr. from previous meal.

(2) After road transport of fasted pigs about 40 miles to bacon factory.

(3) Without applying the allowance for shrinkage on cooling.

(4) As measured from front rib to *pubis symphysis*.

(5) Total weights of flares, fillets and kidneys from both sides.

(6) Based on weights of trimmed sides before curing.

(7) Back fat sampled from gammon end of sides (bung fat).

The results for the group-fed pigs in Table 11 suggest that the pigs on the diets containing the lower levels of protein food gave slightly fatter rashers than the animals on the standard treatment A, but the differences were not statistically significant. The relatively small influence of dietary factors on the production of lean and fat is further illustrated by the results in Table 12 for the area of lean known as the "eye" muscle, the dimensional characteristics of which were not significantly affected by the differences of protein supply in the three treatments. It is further to be noted that the treatment means for the thickness of the back fat opposite the "eye" muscle show no significant differences.

Comments on Table 13

The results for the individually fed pigs show that the differences in respect of protein supply gave rise to no significant differences of confirmation in the pigs as judged on the basis of length, carcass percentage and the weights of the sides expressed as percentages of the carcass weights; nor were the weights of the kidneys, of the flares (a physiological unit of fatty tissue) and the fillets (a physiological unit of muscular tissue) affected significantly by the differences of feeding treatment. The results point to a significant lowering of the iodine value of the bung fat in the case of the pigs on treatment C, but this is to be attributed to the lower amount of fish meal oil consumed by the C pigs rather than to differences in the level of protein. The results for the group-fed pigs are in satisfactory agreement with those for the individually fed pigs, particularly when account is taken of the less sensitive lay-out of this section of the trial (see Table 4).

That the differences in feeding treatment had been without influence on carcass quality as judged by the factory grader is revealed by the following analysis of the payment grades:

	No. of pigs graded	
	A	B
Treatment A	15	5
Treatment B	16	5
Treatment C	14	6

SUMMARY

It has been shown that a diet supplying, in addition to barley meal, weatings and 2% of lucerne meal, 5% of fish meal up to 90 lb. live weight, 2% from 90 to 150 lb. and no protein-rich food from 150 lb. to slaughter at about 200 lb. live weight, will give almost as good results, from the standpoints of mean daily live-weight increase and efficiency of food conversion *over the whole period* as one that provides 10% of fish meal from weaning to 150 lb. and 5% of a protein-rich food such as ex. soya bean meal from 150 to 200 lb. live weight. The small differences noted in the present trial were not statistically significant. It is true that the reduction of the fish meal supply to 5% or less retards to a significant extent the rate of growth up to 90 lb. live weight, but this initial setback, according to the results of this and the writers' previous investigations, tends to be wiped out during the later stages of feeding.

Further, a diet supplying, in addition to cereal, weatings and a small proportion of lucerne meal, as little as 2% of fish meal up to 90 lb. live weight and no protein-rich food at all thereafter has been shown to give results that are not seriously inferior, particularly when viewed against the background of wartime conditions, to those obtained on the standard diet. In this treatment, the protein required for growth beyond 90 lb. live weight was supplied by a diet of barley meal and weatings reinforced by 2% of lucerne meal.

The trial revealed no evidence that the lowering of the supply of protein-rich food to these levels had any disadvantageous effect on the leanness and quality of the bacon carcasses.

The results suggest that a diet made up of cereals and weatings, with a little fresh or artificially dried green food, and supplying 7% of white fish meal (or its equivalent of other protein food) from weaning to 90 lb. live weight, and none at all thereafter, should provide all the protein that is necessary for quick growth and satisfactory carcass quality. This hypothesis has already been tested in further trials, and the results will be published shortly.

In conclusion, the writers take this opportunity of thanking Mr J. Andreasen, of the St Edmundsbury Co-operative Bacon Factory, for granting facilities for carrying out the factory part of the investigation. They also express their indebtedness to Dr J. Wishart for taking charge of the statistical analysis of the results and to Dr E. H. Callow and his

staff for valuable assistance in the factory part of the work. The care of the pigs throughout the feeding trial was in the hands of Mr E. A. Porter, to whom the writers also tender their thanks.

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THE INHERITANCE OF RACHILLA LENGTH IN BARLEY

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(With Eighteen Text-figures)

INTRODUCTION

THE inflorescence in the genus *Hordeum* consists of a series of triads of single-flowered spikelets borne in a spike-like manner on the rachis or inflorescence axis. This axis possesses many nodes and internodes (usually 10–15), and each triad arises alternately on opposite sides of the rachis, thereby giving rise to six longitudinal rows of single-flowered spikelets. Owing to the shortness of the rachis internodes, each spikelet overlaps the one immediately above to a greater or less degree, there being considerable variation in the laxity and density of the inflorescence between certain varieties. In the cultivated forms of barley the middle, or median, spikelet of each triad always possesses a fertile flower, and the spikelet itself is sessile on the rachis. The condition of the lateral spikelets varies according to the botanical form, and in some cases the spikelet is borne on a very short and inconspicuous pedicel. When these lateral spikelets possess hermaphrodite flowers and set grain (as the medians invariably do) the so-called "six-row" and "four-row" barleys result. These forms may be included in the one group *polystichum*. The reduction of the lateral florets to a staminate condition leads to the "two-row" form generally designated as *distichum*, while further reduction in which the laterals are abortive and consist only of sterile glumes results also in the two-row botanical form, but is recognized as a distinct type termed *deficiens*.

The rachilla, or spikelet axis, is relatively insignificant in barley, a condition due presumably to the single-flowered nature of the spikelets. When the grain is detached from the rachis, the rachilla usually breaks away with it, and may be seen lying along the ventral groove of the grain with its base arising from within the folded edges of the lemma where they overlap at the grain base (Figs. 1–3). In certain cases, dependent on the variety and the state of maturity of the ear, the rachilla is left adhering to its node on the rachis when the grain is detached.

In spite of the delicate nature of the rachilla, it is usually quite undamaged when it is removed with the grain, but rough handling of grain samples may cause the rachilla to be broken at its tip, or even to be detached from the grain.

The more or less conspicuous nature of the rachilla as a character of the grain attracted the attention of the early investigators engaged on

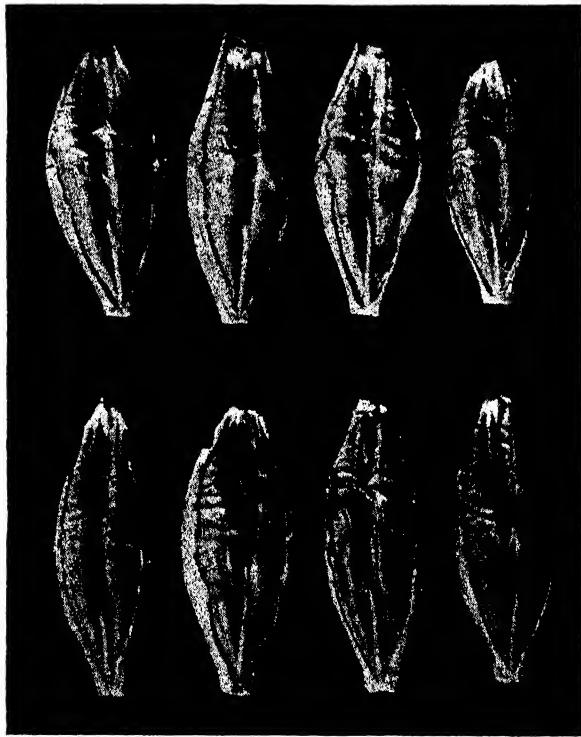


Fig. 1. Upper, Old Scotch common; lower, Old Irish. Both varieties show short "Archer" type rachilla.

the study of varietal characters in cultivated barley, and the type of hair or bristle borne on the rachilla was soon recognized as a clear-cut and distinctive character which could be utilized in varietal classification. Broadly speaking, two conditions of this character are recognized, viz. the long, straight and bristle-like hairs on the one hand, and the short, "woolly" hairs on the other, known respectively as the "Archer" and "Chevallier" types by workers in England, because Archer and Chevallier were the most widely grown varieties in this country at the time of this

early work (Figs. 1-3). The investigations of Atterberg, Neergaard and Bolin established this rachilla character as of fundamental importance in any system of varietal classification of cultivated barley based on grain characters, and it was found subsequently that the type of hair on the rachilla was linked with that of the lodicules, and that a single factor difference explained the inheritance of the "Archer" and "Chevalier" types.



Fig. 2. Upper, Russian I; lower, Long Eared Nottingham. Both varieties show long "Archer" type rachilla.

lier" types. Certain minor differences in the hairiness of the rachilla have been studied by Wiggans (1921), who has utilized these differences for varietal descriptions of agricultural varieties cultivated in the U.S.A.

Bell (1937) has shown that the length of the rachilla is a varietal character of limited value in the classification and identification of agricultural varieties. He measured the rachilla lengths of a large number of varieties, and used the character for varietal descriptions based on grain characters, but he drew attention to the difficulty of establishing

any absolute rachilla length as characteristic of a particular variety owing to the environmental fluctuations. This work suggested that a biometric study of the character would demonstrate the limits of varietal fluctuation, and might establish a useful criterion on which to base

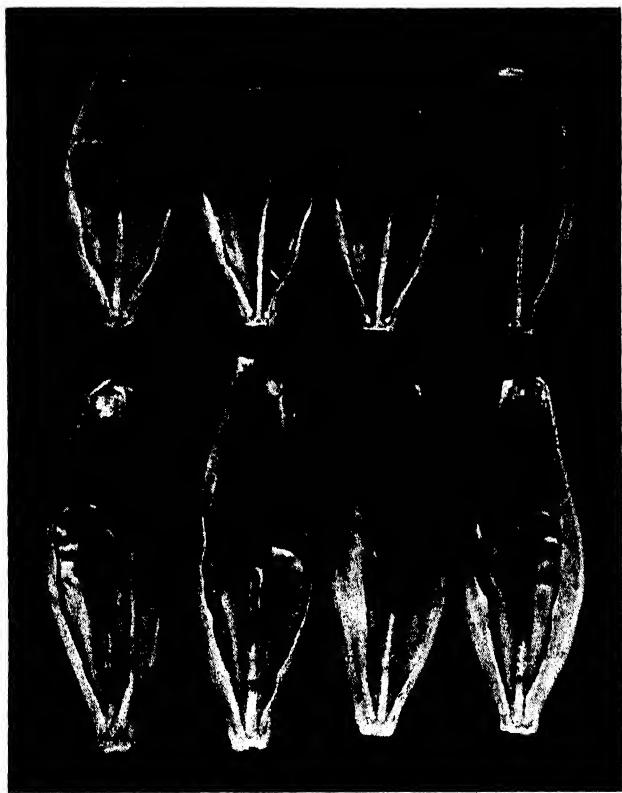


Fig. 3. Upper, Chevallier P.B.1. showing "Chevallier" type rachilla; lower, *Deficiens*, showing modified form of "Chevallier" type rachilla.

varietal measurements. Further, the nature of the character, and the clear-cut differences between varieties with the shortest rachilla on the one hand, and the longest rachilla on the other, indicated that rachilla length might be useful for studying a measurable character from the genetic point of view. This paper deals with the results so far obtained in the investigations which have been interrupted by the war.

RACHILLA LENGTH IN SOME SIX-ROW VARIETIES AND THEIR HYBRIDS

In 1934 Stephens studied the rachilla length in some six-row varieties and their hybrids. The results of this work were submitted for the Diploma in Agricultural Sciences at Cambridge, and have not been published previously.

Stephens commenced his study by measuring every measurable rachilla in each of three ears of the two six-row varieties denoted as Irish six-row and *H. parallelum* (a form of *polystichum*). The object of this preliminary work was to obtain some measure of the fluctuation of the rachilla length within the ears, and the position of each rachilla and the lengths of the corresponding rachis internodes were observed. Tables 1 and 2 give the measurements obtained for one ear of each variety, and may be taken as typical of the other ears. It may be observed that as the internodes lengthen from the base of the ear to some intermediate position, and then taper away to the tip of the ear, so also do the rachilla lengths vary. Therefore the distribution of the rachilla lengths along the ear follows the same general trend as the internode lengths, long rachillas tending to occur in the same region of the ear as do long internodes. The association is of a general nature, and obviously is not so close that the longest and shortest rachillas are invariably associated respectively with

Table 1. Internode and rachilla length (in mm.) in one ear of the variety Irish six-row

Base of ear	Length of internodes	Length of median grain rachillas	Length of lateral grain rachillas	
	3.25	3.75	—	—
	3.75	3.75	—	4.25
	3.75	4.25	4.25	4.25
	4.25	4.00	4.75	4.25
	4.25	4.25	4.50	4.25
	4.00	4.00	4.50	4.50
	3.75	4.25	—	—
	3.75	4.00	4.25	4.50
	3.50	3.75	4.50	4.50
	3.50	4.25	—	4.25
	3.50	—	4.50	—
	3.50	—	4.25	4.00
	3.50	3.75	4.50	4.00
	3.50	3.75	4.00	4.25
	3.50	4.00	4.25	4.25
	3.25	3.75	3.75	4.25
	3.25	4.00	4.25	4.00
	3.25	3.25	—	4.25
	3.00	3.25	3.75	3.50
	3.00	3.25	4.00	4.00
	3.25	3.75	3.75	3.50
	3.25	3.50	—	—

Apex of ear

Table 2. *Internode and rachilla length (in mm.) in one ear of the variety H. parallellum*

Base of ear	Length of internodes	Length of median grain rachillas	Length of lateral grain rachillas	
↑	2.00	4.00	4.50	4.50
	2.50	4.00	4.00	4.00
	2.75	3.75	4.00	4.25
	2.75	3.75	4.25	4.25
	3.00	3.50	4.25	4.25
	3.00	4.00	3.50	4.00
	3.00	3.75	4.00	3.75
	3.00	3.75	4.00	3.50
	3.25	3.50	4.00	3.75
	3.00	3.75	4.00	4.00
	3.00	3.50	4.25	3.50
	3.00	3.75	—	4.00
	3.00	3.25	4.25	3.75
	3.00	3.50	—	4.00
	3.25	3.50	—	4.00
	3.00	3.50	3.50	3.50
	3.00	3.00	4.00	3.00
	3.00	3.25	3.75	4.00
	3.00	3.00	3.75	3.50
	2.75	3.25	3.75	3.25
	2.75	3.00	3.50	3.25
	2.75	3.00	3.75	3.75
	2.75	3.00	3.50	4.00
	2.75	3.00	3.25	3.50
	2.75	2.75	3.25	3.50
	2.75	2.75	3.50	3.25
	2.75	2.75	3.50	3.50
	2.75	2.75	—	3.25
	2.75	2.75	—	3.25
↓				
Apex of ear				

the longest and shortest internodes. Further, a change in the length from one internode to the next is not necessarily accompanied by a corresponding change in the rachilla, and breaks occur in the continuity of progressive lengthening and shortening. Two other points of interest are worth noting from these figures. First, the length of the rachilla of the median and lateral grains is at least as long as, and is generally longer than, the corresponding internode. Secondly, in any triad of grains, the rachillas of the lateral grains are generally longer than that of the median, but the laterals themselves may show considerable variation when comparing the individuals of each pair. From the above results, therefore, it is important if samples of grain from six-row varieties are being compared, that the same proportion of lateral and median rachillas should be measured in each, and Stephens adopted the method of measuring equal numbers of each type.

In a six-row variety, therefore, there is every reason to believe that fluctuation of rachilla length can be expected owing to the position of

the grain on the ear, and that small medians from the tip will have the shortest rachillas, but that the basal grains, although often associated with short internodes, will not necessarily have short rachillas. Sufficient ears were not examined to obtain any evidence concerning the fluctuation between the ears.

It has been stated previously that evidence obtained by Bell indicated that considerable fluctuation may be expected from season to season. Stephens studied this fluctuation by measuring bulk samples of 240 grains (120 medians and 120 laterals) of the three varieties Irish six-row, *H. parallelum* and Bigo, grown in two separate years, 1932 and 1933. The results are given in Table 3.

Table 3

Variety	1932			1933			$\bar{X}^2 > \bar{X}^1$		
	\bar{X}^1	mm.	n	S.E.	\bar{X}^2	mm.	n	S.E.	
<i>H. parallelum</i>	2.78	240		± 0.0412	3.48	240		± 0.0201	Signif. $P=0.05$
Irish six-row	3.60	240		± 0.0325	3.77	240		± 0.0287	"
Bigo	3.85	240		± 0.0416	4.16	240		± 0.0354	"

Each variety shows a significant increase in the length of the rachilla in 1933 as compared with 1932, but the proportional increase is different for each of the three. It is interesting to notice that the greatest interseasonal fluctuation is not associated with the variety possessing the longest rachilla, viz. Bigo, although this variety is characterized by the greatest intraseasonal fluctuation in both years.

These studies indicate that for genetic study, or varietal comparisons of rachilla lengths the material should be grown under as similar conditions as possible. In six-row varieties the difference between the lengths of the rachillas of the median and lateral grains is a possible source of error, because not only are the rachillas of lateral grains longer than those of medians, but the difference between the laterals and medians varies in individual varieties. In two-row varieties the question of lateral grain does not arise, and fluctuation is confined to position on the ear and environmental conditions.

Two crosses involving the six-row varieties mentioned above were studied by Stephens. The parents and the F_1 's were grown in 1932, and the parents and F_2 's in 1933. Bulk samples of 240 grains (120 medians and 120 laterals) were measured in the case of the parents and the F_1 's, while fifty plants were taken at random in the F_2 's and fifty grains (twenty-five medians and twenty-five laterals) measured for each plant. The F_2 's consisted, therefore, of 2500 grains.

A. *H. parallelum* \times IRISH SIX-ROW

H. parallelum possesses the "Archer" type of rachilla, and Irish six-row the "Chevallier" type, the former being considerably the shorter of the two. Table 4 gives the parental and F_1 means in 1932, and Fig. 4 represents the curves of the three populations. The F_1 rachillas were all of the "Archer" type and the mean was significantly longer than the longer parent, Irish six-row, which in turn was significantly longer than the shorter parent, *H. parallelum*.

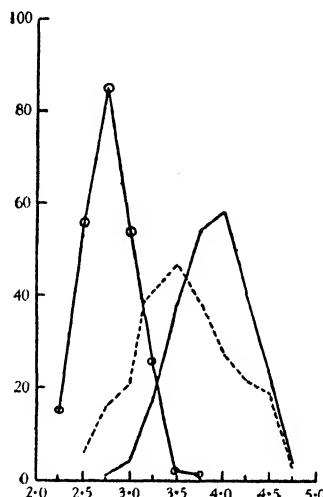


Fig. 4. *H. parallelum* \times Irish six-row. O—O "Archer" type parent; - - - "Chevallier" type parent; — F_1 .

Table 4

Variety or generation	Mean mm.	n	S.E.
<i>H. parallelum</i> (“Archer” type)	2.78	240	± 0.0142
Irish six-row (“Chevallier” type)	3.60	240	± 0.0325
F_1 (“Archer” type)	3.89	240	± 0.0232

The F_2 and parents were grown in the following year when, as mentioned previously, rachilla lengths were longer than in 1932. Stephens observed that the "Chevallier" rachilla types of the F_2 were longer than the "Archer" types, and he consequently kept the types separate in the F_2 , data for which are given in Table 5.

As in 1932, the rachilla length of Irish six-row was significantly longer than that of *H. parallelum*, although the difference was not the

Table 5

Variety or generation	Mean			
	mm.	n	S.E.	σ
Irish six-row ("Chevallier") type	3.77	240	± 0.0287	0.444
<i>H. parallellum</i> ("Archer" type)	3.48	240	± 0.0201	0.312
F_1 ("Chevallier" type)	4.12	650	± 0.0162	0.412
F_2 ("Archer" type)	3.69	1850	± 0.0096	0.415

same in the two years. The treatment of the "Chevallier" and "Archer" types as separate populations in the F_2 shows that the former is significantly longer than the latter, and that each is significantly longer than its corresponding parental type. The "Chevallier" rachilla type is recessive to the "Archer" type, and the standard deviation of the former type in the F_2 is slightly less than the latter, and also appreciably smaller than the parental "Chevallier" type. In contrast to this latter relation of the "Chevallier" types, it is important to notice that the standard deviation of the F_2 "Archer" population is considerably larger than that of the "Archer" parent. The distinct nature of the two F_2 populations is emphasized by the longest and shortest F_2 plant means in each, those for the "Archer" type being 4.145 and 3.350 mm. and for the "Chevallier" type 4.390 and 3.880 mm. respectively. These conditions are emphasized further by the graphs of Figs. 4 and 5 which show the distributions for the parents, F_1 and F_2 populations. On the other hand, if the F_2 is taken as a single population (Fig. 6) a unimodal distribution is obtained with a mean of 3.799 mm., which is almost identical with that of the longer parent.

B. *H. PARALLELUM* \times BIGO

In this cross *H. parallellum* is again hybridized with a variety possessing a significantly longer rachilla of the "Chevallier" type. The data showing the parental and F_1 means are given in Table 6.

Table 6

Variety or generation	Mean			
	mm.	n	S.E.	
<i>H. parallellum</i> ("Archer" type)	2.78	240	± 0.0142	
Bigo ("Chevallier" type)	3.85	240	± 0.0416	
F_1 ("Archer" type)	3.91	240	± 0.0275	

The mean of the F_1 in this cross is not significantly longer than the mean of the longer parent, and in this respect differs from the *H. parallellum* \times Irish six-row cross. It will be seen that this lack of significant increase in the F_1 mean is associated with a larger difference in the means of the parents than was apparent in the previous cross. The distributions

of the rachilla lengths of the parents and the F_1 of the $H. parallelum \times$ Bigo cross are given in Fig. 7.

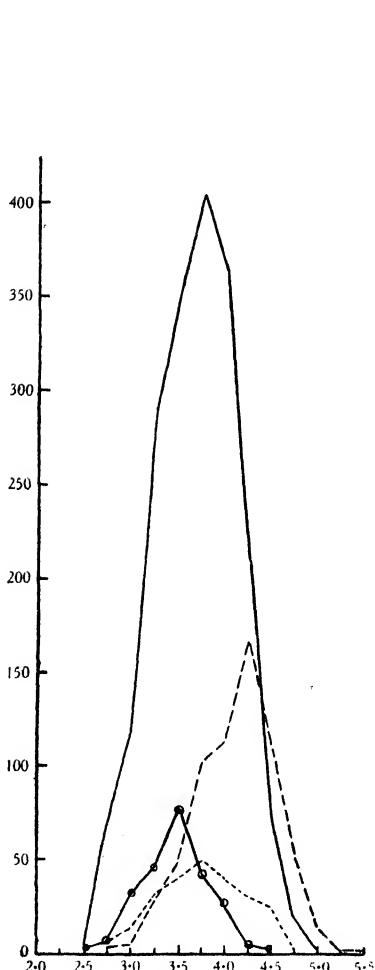


Fig. 5.

Fig. 5. $H. parallelum \times$ Irish six-row. $\circ-\circ$ "Archer" type parent; $-\cdots-$ "Chevallier" type parent; $-$ "Archer" types of F_1 ; $-\cdots-$ "Chevallier" types of F_1 .

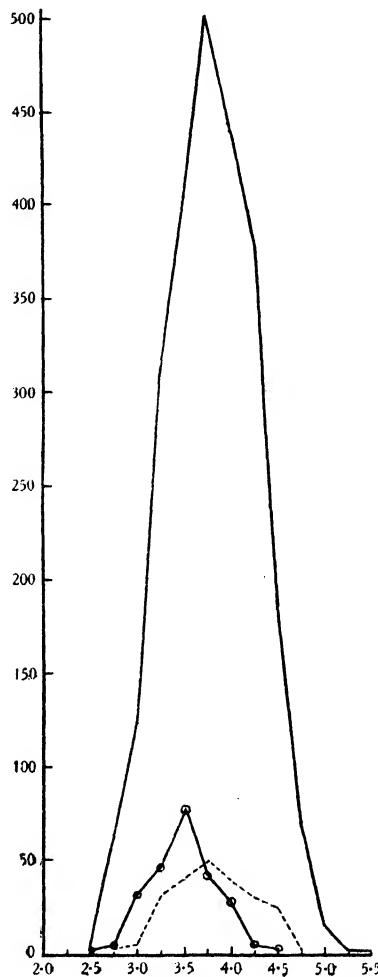


Fig. 6.

Fig. 6. $H. parallelum \times$ Irish six-row. $\circ-\circ$ "Archer" type parent; $-\cdots-$ "Chevallier" type parent; $-$ F_1 .

The data for the parents and the F_2 are given in Table 7, and although there is a marked increase in the rachilla lengths of the two parents compared with the previous year, the difference continues to be significant.

Table 7

Variety or generation	Mean mm.	n	S.E.	σ
<i>H. parallelum</i> ("Archer" type)	3.48	240	± 0.0201	0.312
Bigo ("Chevallier" type)	4.16	240	± 0.0354	0.548
F_2 ("Archer" type)	3.92	1950	± 0.0119	0.524
F_2 ("Chevallier" type)	4.23	550	± 0.0228	0.535

The difference between the means of the "Archer" and "Chevallier" F_2 populations is statistically significant as in the previous cross, but although the F_2 "Archer" population is significantly longer than the

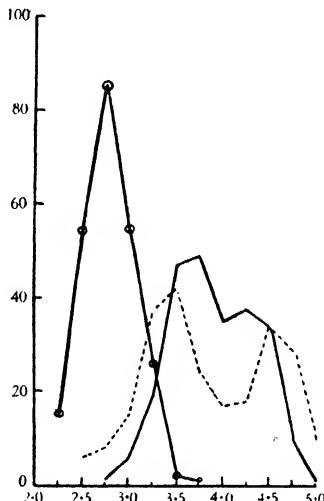


Fig. 7. *H. parallelum* \times Bigo. ○—○ "Archer" type parent;
- - - "Chevallier" type parent; — F_1 .

"Archer" type parent (*H. parallelum*), the F_2 "Chevallier" population is not significantly longer than the "Chevallier" type parent, although there is a noticeable increase. It is interesting to remember that the F_1 of this cross in the previous year was not significantly longer than the longer parent, and this lack of significant increase in the F_1 , and in the "Chevallier" types in the F_2 , constitutes a consistent difference between the observations of this and the previous cross.

The relation between the standard deviations of the F_2 populations and the parents is similar to the previous cross, the most interesting considerations being that the F_2 "Archer" population possesses a standard deviation significantly greater than the "Archer" type parent, whereas the standard deviation of the F_2 "Chevallier" population is, if

anything, slightly below that of the "Chevallier" type parent. The distinct nature of the two F_2 populations is illustrated again by the figures for the extreme plant means of rachilla length, those for the "Archer" types being 4.425 and 3.325 mm., and for the "Chevallier"

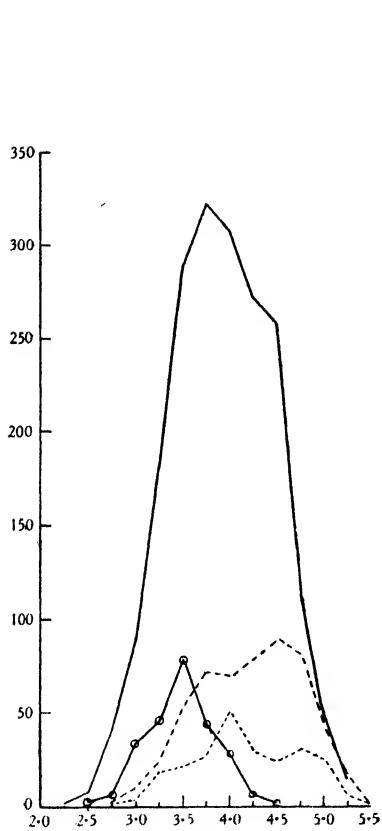


Fig. 8.

Fig. 8. *H. parallelum* \times Bigo. ○—○ "Archer" parent; - - - "Chevallier" parent; —— "Archer" type F_2 ; —— "Chevallier" type F_2 .

Fig. 9. *H. parallelum* \times Bigo. ○—○ "Archer" type parent; - - - "Chevallier" type parent; —— F_2 .

types being 4.470 and 4.055 mm. The distributions in the F_1 , and in the F_2 considered as separate populations and in bulk, are given in Figs. 7-9. It may be seen that although there are the same general trends as in the previous cross, the mean of the bulked F_2 is 3.983 mm., which is intermediate between the two parents, though tending towards the

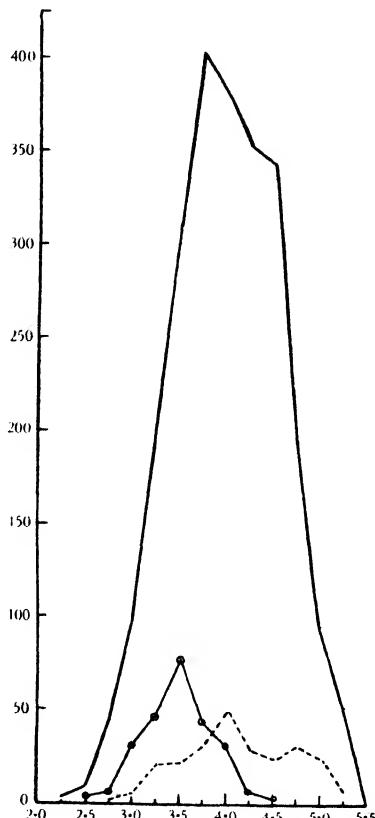


Fig. 9.

longer one, instead of being identical with the longer parent as in *H. parallellum* \times Irish six-row. This condition follows naturally from the observations of the behaviour of the F_1 and the F_2 "Chevallier" population in the present cross, in which the means were not significantly greater than the parent with the long rachilla, whereas they were so in the previous cross.

THE CORRELATION OF RACHILLA LENGTH WITH INTERNODE LENGTH

Observations made by Bell (1937) suggested that there was a correlation between rachilla length and internode length. From measurements made on over 50 varieties of two-row barley, Bell concluded that dense ear varieties are characterized by possessing short rachillas, the shortest rachilla forms being found in the dense ear group. Subsequent work by Stephens on the three six-row varieties already mentioned (pp. 250-2), and on two lax ear and two dense ear varieties of the two-row class, have substantiated this view, while the F_1 's of two crosses between six-row and two-row forms may be considered also. In comparing six-row and two-row varieties it was decided to consider only median grains, and the mean rachilla length of each variety was based on 120 grains, and the mean internode length was obtained by measuring ten internodes in each of twelve ears. The data, although based on admittedly small populations of varieties which in some ways are not typical, offer further evidence of the association under consideration (Table 8). However, it cannot be said that dense ear varieties possess invariably shorter rachillas than lax ear varieties, but it should be noted that the variety Spratt has been shown by hybridization to be a somewhat anomalous dense ear type, while *Deficiens* II may not be comparable owing to the complete suppression of the laterals. Nevertheless, considering the lax ear varieties as a group, there is a strong indication that median rachilla length is correlated with internode length, while in these forms the internode length in all cases is greater than the rachilla length. In the dense ear group there is also a correlation between the two measurements, but in this case the internode length of any variety is less than its median rachilla length.

Further support of this correlation between internode length and rachilla length was obtained in a study of fifty F_2 plants from the cross *Deficiens* II \times Brage. In this study only the main ear of each F_2 plant was measured, and the mean rachilla length for each internode-length class was calculated (Table 9). Again, the smallness of the population

militates against any very definite statement being made, but when all the evidence from the different sources is considered there are strong grounds for the conclusion that long internodes are associated with long rachilla lengths.

Table 8

Variety or generation	Internode length mm.	Median rachilla length mm.
Lax ear group:		
<i>F</i> ₁ Irish six-row × Spratt-Archer	4.77	4.42
<i>F</i> ₁ <i>H. parallellum</i> × Spratt-Archer	4.34	3.93
Bigo (six-row)	4.26	3.76
Brage (two-row)	3.92	3.72
Spratt-Archer (two-row)	3.89	3.60
Irish six-row	3.77	3.53
Dense ear group:		
<i>Deficiens</i> II (two-row)	3.38	3.73
Spratt (two-row)	2.82	3.57
<i>H. parallellum</i> (six-row)	2.77	3.30

Table 9

Internode class mm.	Average mean rachilla length in the class mm.	No. of plants
5.00	4.27	1
4.75	4.51	8
4.50	4.30	13
4.25	4.07	10
4.00	3.91	16
3.75	3.65	2

These preliminary studies, which have been described in the first part of this paper, have shown that the following statements are tenable in connexion with the length of the rachilla in barley:

(1) Rachilla length is a varietal character of sufficient stability for genetic study.

(2) Rachilla length varies with the position on the ear and appears to be correlated with the internode length. In six-row varieties, the rachilla length varies with the median or lateral position within the spikelet triad.

(3) Rachilla length is associated with the type of hairs borne on the rachilla, i.e. whether of the "Archer" or "Chevallier" type.

(4) Rachilla length fluctuates to such a large degree with the growing conditions, that comparisons between varieties or populations, or measurements of different generations in genetic work, should be made with material grown under as similar conditions as possible.

FURTHER EVIDENCE CONCERNING THE INHERITANCE OF RACHILLA LENGTH IN SIX-ROW HYBRIDS

In 1935 a number of hybrids between six-row varieties was studied to see whether any evidence could be obtained of a possible heterotic effect, or of the recombination of complementary factors. These hybrids were not made originally for the study of rachilla length, and only the F_1 generation was measured. In all cases bulk samples of grain were measured, and the results are presented in Table 10.

Table 10

Variety and hybrid	Mean mm.	S.E.	Remarks
$H. parallelum$ ("Archer" type)	2.57	±0.026	
Kors ("Chevallier" type)	3.36	±0.032	Significantly longer
F_1 ("Archer" type)	3.18	±0.030	than $H. parallelum$
Bigo ("Chevallier" type)	3.83	±0.045	
$H. praecox$ ("Archer" type)	4.26	±0.050	Significantly longer
F_1 ("Archer" type)	4.55	±0.035	than Bigo Significantly longer
Irish six-row ("Chevallier" type)	3.10	±0.057	than $H. praecox$
$H. praecox$ ("Archer" type)	4.26	±0.050	Significantly longer
F_1 ("Archer" type)	4.39	±0.003	than Irish six-row Significantly longer
			than $H. praecox$

In each of these crosses one parent possessed an "Archer" type rachilla, and the other parent a "Chevallier" type rachilla. In the first cross the "Chevallier" type parent and the "Archer" type F_1 were significantly longer than the "Archer" type parent, there being no suggestion of an increase of the rachilla length of the F_1 beyond that of the longer parent.

The two other crosses differ from any of the six-row crosses yet considered in that the "Chevallier" type parent possesses a rachilla significantly shorter than that of the "Archer" type parent. In both cases the F_1 rachilla, which is of the "Archer" type, is significantly longer than the longer parent.

It appears, therefore, from these three crosses, and the two previously described, in which one parent possesses a rachilla significantly longer, and of a different type than the other parent, that the mean of the F_1 may be as long as, or significantly longer than, the mean of the longer parent. These two conditions of the F_1 can result when either the "Archer" or "Chevallier" type parent is the longer, and in no case has the hybridization resulted in an F_1 with a mean intermediate between

those of the two parents. Further, it must be supposed that in three of the five crosses a possible heterotic effect has been obtained.

The above conditions are not confined to six-row \times six-row crosses, however, similar results having been obtained when *Deficiens* II was hybridized with the two six-row varieties, *H. parallelum* and Bigo, and the two-row variety Brage. The mean rachilla length of *Deficiens* II was 2.089 mm., which was significantly less than that of *H. parallelum* (2.57 mm.), Bigo (3.834 mm.) and Brage (2.741 mm.), while the F_1 's of the three crosses were 3.0175, 3.891 and 3.523 mm. respectively. Thus, once more there are the two conditions of the F_1 being either significantly greater than, or equal to, the longer parent. It must be realized also that Brage has a "Chevallier" type of rachilla, while the rachilla of *Deficiens* II is probably also of that type although the hairs are somewhat longer (Fig. 3). The cross *Deficiens* II \times Brage involves two parents with different ear types (although both have only two rows of grains) and a possible minor difference in rachilla type, and the resulting F_1 is significantly greater than the longer parent. A similar condition of the F_1 is found in the cross *Deficiens* II \times *H. parallelum*, where the parents differ in ear type and rachilla type. The F_1 of *Deficiens* II \times Bigo fails to show a significant increase above the longer parent, although there is the major difference of ear type and the minor difference of rachilla type. This may be due to the fact that Bigo has abnormally longer rachillas to the laterals, and their inclusion has increased the mean abnormally. It must be remembered that there are no lateral grains on the ears of F_1 plants when a six-row and a *deficiens* form are hybridized. But regardless of whether the F_1 is as great as, or greater than, the long parent, all these crosses have one common feature, the dominance of long rachilla.

INHERITANCE OF RACHILLA LENGTH IN TWO-ROW HYBRIDS

The evidence presented in the foregoing sections of this paper shows clearly that several factors can complicate any attempt to analyse genetically the mode of inheritance of the length of the rachilla in barley. These factors can be considered generally under two composite headings, viz. (1) the environment, (2) the material selected as parents. In order to remove these complicating factors as far as possible certain precautions should be taken.

(1) *The environment.* It has been shown by studying the fluctuations from season to season that large environmental effects must be expected. These effects could be counteracted only if the parents, F_1 , F_2 , and F_3

were grown in the same season and in one locality. Strictly speaking it is impossible to do this, because it would be necessary to make three distinct hybridizations, and there would be some objection to interpreting an F_1 in terms of an F_2 which had not been derived from it. The same objection holds for the relation between the F_2 and the F_3 , while in this case there is the added difficulty that it would be impossible to grow an F_3 which had been derived from selected F_2 plants which were being grown in the same year. It appears, therefore, that the three generations must be grown in different seasons, and a measure of the seasonal fluctuation obtained by comparing the parental data of each sowing. This is the procedure which has been adopted in the hybrids to be described in this section.

(2) *The parents.* The obvious and outstanding causes of complication with regard to the parents are in relation to botanical type and rachilla type. The botanical type is important in so far as it affects the morphology of the ear, and the preliminary investigations described in the beginning of this paper showed that the six-row or two-row condition, and the density of the ear, as measured by the internode length, are complicating factors. Other botanical characters may also be important, but the material employed in the present studies offered no other major botanical differences as far as the ear is concerned. The rachilla type, as defined by the type of hairs or bristles, must be considered of sufficient importance in its effect on the inheritance of rachilla length as to justify the avoidance of introducing the complication of having two types in genetic work which is attempting a straightforward analysis of the length character.

In the light of the above botanical considerations, it was decided to eliminate as far as possible the sources of complication by choosing parents with similar ear characters and rachilla type. Accordingly, varieties were selected from the two-row, lax ear, "Archer" rachilla group, and on the basis of the survey made by Bell of these varieties, suitable forms were chosen representing the extremes of rachilla length. It was found by Bell that three varieties, viz. Old Irish, Scotch Common and Swanneck, formed a group with extremely short rachillas, while the varieties Long Eared Nottingham and Russian II were quite distinct in their extremely long rachillas. Consequently, these five varieties formed the basis of the later work now to be described (Figs. 1 and 2).

Samples of grain of each variety were taken from available bulks from autumn sown and spring sown material, in order to confirm the measurements obtained in the preliminary survey of the varieties and to

obtain some measure of the parental fluctuation. At least 300 grains were measured for each variety, while in some cases the number reached 500 where sufficient material was available. The mean rachilla length for the two sowings of each variety are given in Table 11, and the frequency distributions in Figs. 10-12.

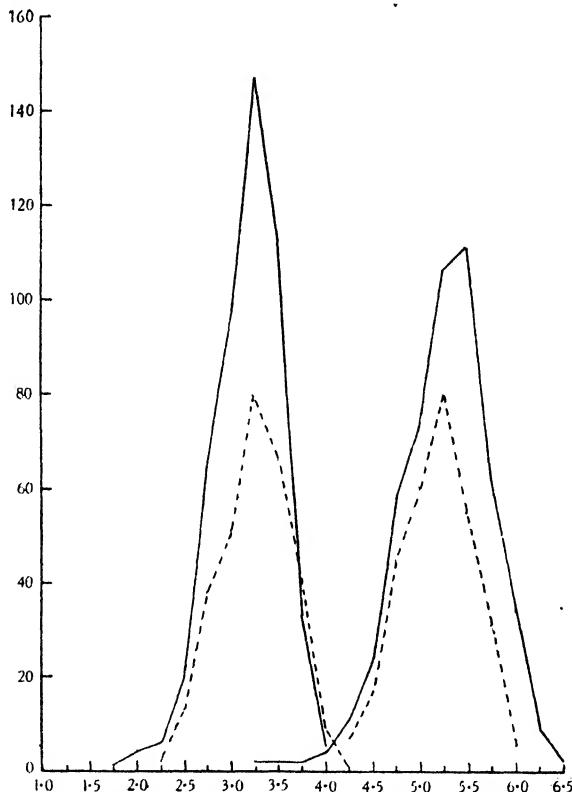


Fig. 10. Short rachilla = Old Irish; long rachilla = Long Eared Nottingham.
— spring sown; - - - autumn sown.

Table 11

Variety	Spring sown	Autumn sown
	mm.	mm.
Swanneck	2.758	2.494
Scotch Common	3.034	2.969
Old Irish	3.174	3.253
Long Eared Nottingham	5.258	5.169
Russian II	5.137	5.183

The above data were obtained by one worker, but subsequent measurements were made by two workers and it was considered desirable to

ensure that each was measuring with a similar precision. Consequently a comparison was made on the variety Scotch Common (Table 12), and the results obtained are indicative of a very small personal error. Indeed, it is improbable whether the same worker making two separate sets of measurements of the same material would reach a higher degree of accuracy.

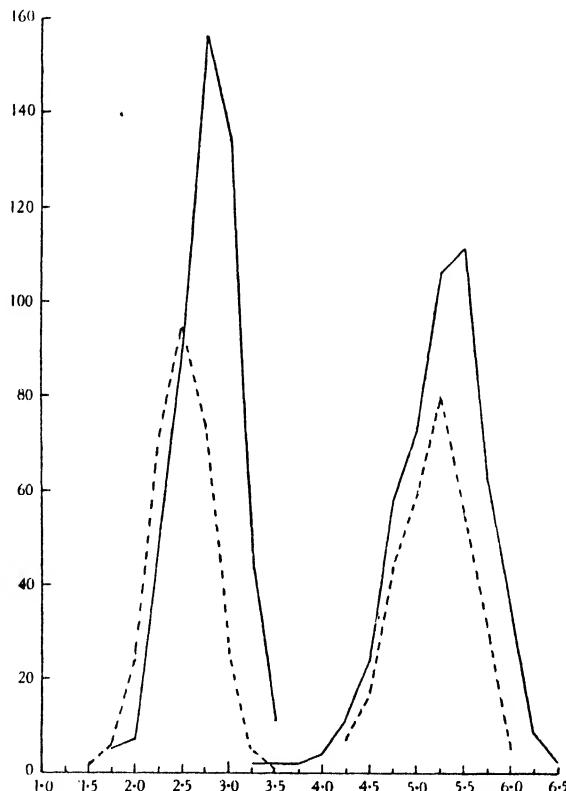


Fig. 11. Short rachilla = Swanneck; long rachilla = Long Eared Nottingham.
— spring sown; - - - autumn sown.

Table 12

	Worker A	Worker B
Autumn sown	2.992 mm. (300 grains)	2.935 mm. (200 grains)
Spring sown	3.039 mm. (300 grains)	3.025 mm. (200 grains)

The means of the autumn and spring sown material indicate a relatively low value for the fluctuation of any one variety, and a comparison of the varietal means shows that there is a large and obvious difference between the short rachillas of the first three varieties on the

one hand, and the long rachillas of the last two varieties on the other. These varietal differences are illustrated even more clearly in Figs. 10-12 where the distributions of a long rachilla and short rachilla variety are contrasted. It may be seen from these curves that there is practically

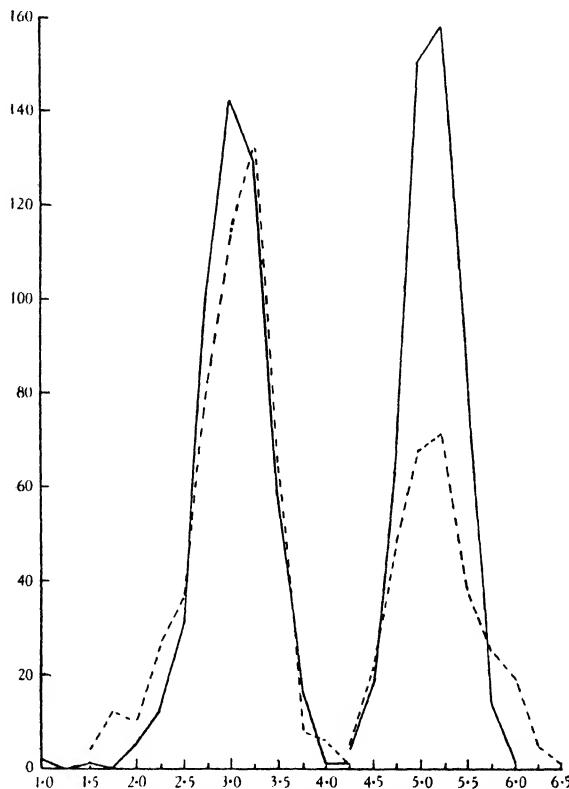


Fig. 12. Short rachilla = Scotch Common; long rachilla = Russian II.
— spring sown; - - - autumn sown.

no overlapping of the contrasting parental curves, and the distribution for any one variety is highly satisfactory for a measurable character (see also Figs. 1-3).

On the basis of these data the following crosses were made:

- (1) Long Eared Nottingham \times Old Irish.
- (2) Long Eared Nottingham \times Swanneck.
- (3) Long Eared Nottingham \times Scotch Common.
- (4) Scotch Common \times Old Irish.
- (5) Long Eared Nottingham \times Russian II.

The first three crosses involve the same long rachilla parent and three different short rachilla parents. Analysis of these three crosses should supply straightforward evidence of the number of factors involved when the extremes of rachilla length are manifested. The data should show also something of the genetic constitution of the four parents. Crosses 4 and 5 were made with the object of obtaining evidence of the presence of complementary factors by the hybridization of varieties possessing similar rachilla lengths of the extreme types.

The method of measurement for these crosses was similar to that employed for the previous material described in this paper. Some attempt was made to measure more accurately than to the nearest 0.25 mm., but this was abandoned because it was not considered practicable from the point of view of time and with the simple apparatus of a mounted dissecting lens and a steel rule.

The measurements of the F_1 's and the parents grown in the same year were made on 500 grains of each particular lot drawn at random from bulked samples of threshed grain. A certain amount of selection was inevitable because damaged rachillas had to be discarded. All subsequent measurements were made, however, on a fixed number of grains taken from individual plants, from which the mean rachilla length was calculated, and consequently the plant became the unit of the populations instead of the individual grain. It was found that twenty grains per plant gave as consistent results as did fifty grains, and the former figure was consequently considered sufficient for the representation of each plant. The spacing of all plants at 2 in. in the row and 6 in. between the rows ensured that, except in the case of damage, there was an ample number of grains from each plant. Indifferent germination and plant establishment in some populations led to incomplete population representation and increased plant spacing, while in certain cases the growth of the plants was poor. Where it is considered necessary, facts of this nature will be mentioned in discussing the data.

THE F_1 GENERATIONS

The summarized data for each of the five F_1 generations with the respective parents are given in Table 13 and the population curves in Figs. 13-17. The intermediate condition of the F_1 's in all cases except in cross 4 can be seen from inspection of both forms of evidence, but is most obvious in the curves showing the results obtained by hybridizing long and short rachilla parents (crosses 1, 2 and 3). In none of these

Table 13

	Mean in mm.	Variance	n
(1) Long Eared Nottingham	5.258 \pm 0.022	0.24279	500
Old Irish	3.174 \pm 0.016	0.12623	500
F_1 L.E.N. \times Old Irish	4.263 \pm 0.015	0.11164	500
Parental mean	4.216 \pm 0.027		
(2) Long Eared Nottingham	5.258 \pm 0.022	0.24279	500
Swanneck	2.758 \pm 0.015	0.10484	500
F_1 L.E.N. \times Swanneck	4.075 \pm 0.017	0.14737	500
Parental mean	4.058 \pm 0.026		
(3) Long Eared Nottingham	5.258 \pm 0.022	0.24279	500
Scotch Common	3.034 \pm 0.017	0.13973	500
F_1 L.E.N. \times Scotch Common	4.363 \pm 0.018	0.16050	500
Parental mean	4.146 \pm 0.028		
(4) Scotch Common	3.034 \pm 0.017	0.13973	500
Old Irish	3.174 \pm 0.016	0.12623	500
F_1 Scotch Common \times Old Irish	3.207 \pm 0.016	0.12370	501
Parental mean	3.104 \pm 0.023		
(5) Russian II	5.136 \pm 0.013	0.08246	500
Long Eared Nottingham	5.258 \pm 0.022	0.24279	500
F_1 L.E.N. \times Russian II	5.151 \pm 0.015	0.11022	500
Parental mean	5.197 \pm 0.026		

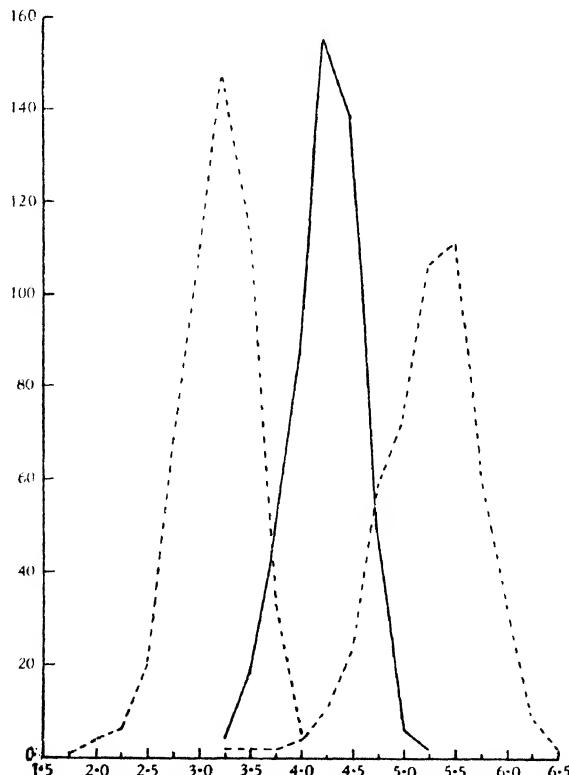


Fig. 13. Long Eared Nottingham \times Old Irish: parents (----) and F_1 (—).

three crosses does the mean of the F_1 equal or exceed that of the longer parent, a condition which is in marked contrast to the results obtained in the previous crosses described. Hybridization of two long rachilla varieties also results in an intermediate condition for the F_1 , but hybridization of two short rachilla varieties gives an F_1 value significantly

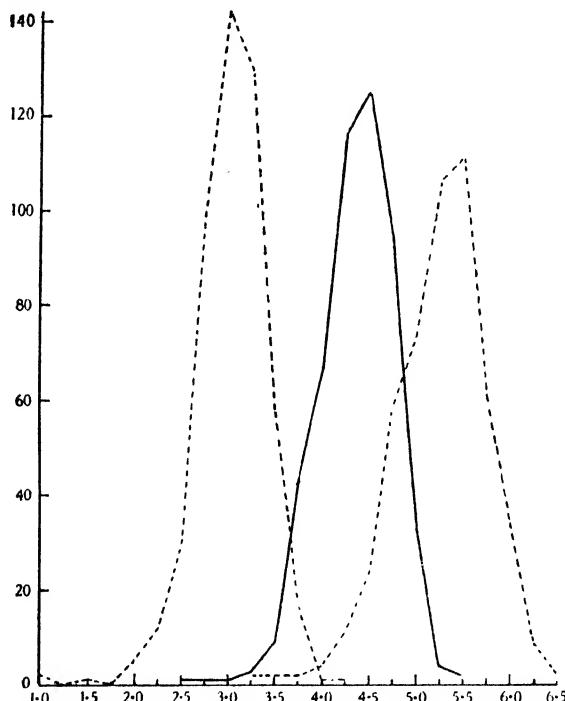


Fig. 14. Long Eared Nottingham \times Scotch Common: parents (----) and F_1 (—).

greater than the mean of the parents. Consideration of the five crosses shows that in two of them (numbers 3 and 4) the mean of the F_1 differs significantly from the mean of the means of the two parents. Both of these crosses include Scotch Common as one of the parents, a fact which may be of significance in indicating some genetic characteristic of this variety unless the value obtained for its mean rachilla length is longer than the true value. On the other hand, the differences between the F_1 means, and the means of the parental means, are small in these two crosses and may be due to environmental conditions, the exact interpretation being a matter of the value attached to the conception of statistical significance of this size in the elucidation of this particular

form of data. At all events the question could be settled only by further study, and in the opinion of the authors should not be stressed. The really important result of the F_1 data from these five crosses is the intermediate condition of the means of four compared with the respective parents. The small values for the standard errors and variances of the F_1

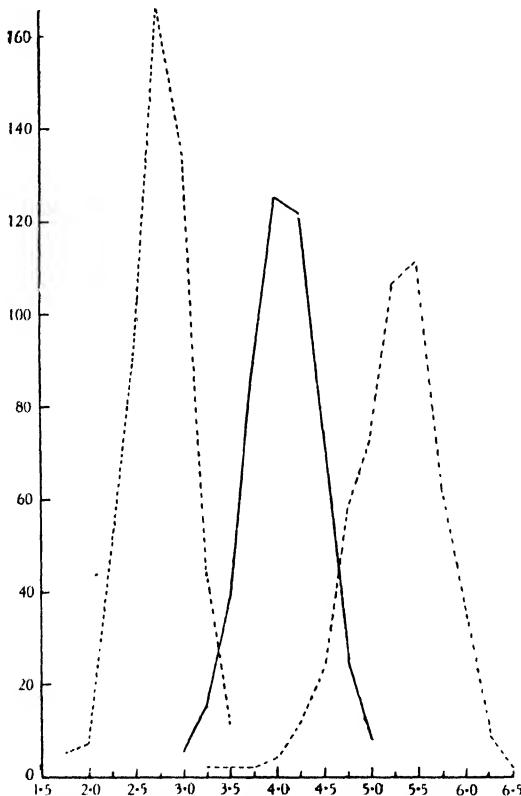


Fig. 15. Long Eared Nottingham = Swanneck: parents (----) and F_1 (—).

populations are also worth mention in obtaining as complete a picture as is possible of the facts available, because the F_1 grain sample was obtained from a smaller number of plants than were the parental grain samples:

THE F_2 GENERATION

All five F_2 generations were grown in the following year with the parents, and the material harvested and threshed as single plants. The amount of material available, and the time involved in the work of

measuring, made it necessary to make some selection in continuing the study. Accordingly, it was decided to choose a cross which would be most likely to give useful information on the direct question of the genetic interpretation of rachilla length, and to use the other crosses for supplementing the main data if the opportunity arose. It was considered that

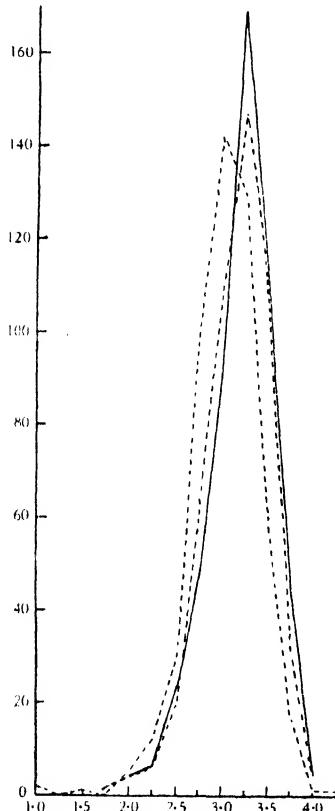


Fig. 16.

Fig. 16. Scotch Common \times Old Irish: parents (----) and F_1 (—).

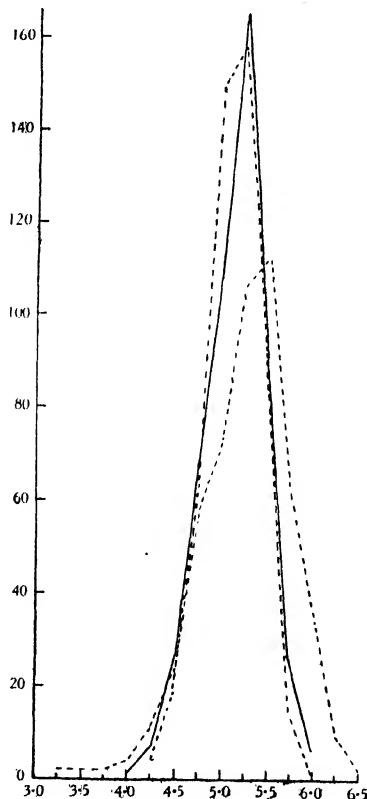


Fig. 17.

Fig. 17. Long Eared Nottingham \times Russian II: parents (----) and F_1 (—).

Long Eared Nottingham \times Old Irish could be regarded as typical of the condition in which the hybridization of a long and a short rachilla variety resulted in the mean of the F_1 population being equal to the mean of the means of the parents, and therefore offering a condition which it was essential to interpret as a basis for the genetic analysis of rachilla length.

The evidence from the F_2 and F_3 generations therefore is based on a study of this one cross.

Table 14 gives the frequency distributions and calculated statistics of the F_2 and the parents grown in the same year, and Fig. 18 shows the frequency distributions. It should be noted that, in Table 14, N refers

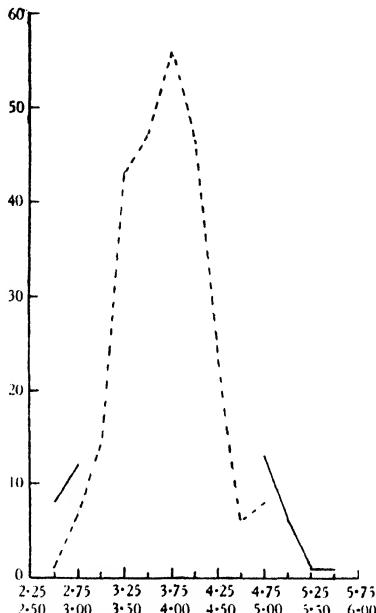


Fig. 18. Long Eared Nottingham \times Old Irish: parents (—) and F_2 (----).

to the number of plants in each of which twenty rachillas were measured. Therefore the number of rachillas on which this F_2 data are based is 5100, while in the parents the numbers were 400 and 420 respectively. It has not been considered necessary to include the rachilla populations for each plant because the space necessary would be unwarranted. Consequently, the distributions of the means of the plants given in Table 14 must be sufficient to illustrate the population characteristic.

The mean rachilla lengths of the parents, based on the means of twenty and twenty-one plants respectively, are lower than in the previous year when the means were calculated on a random grain sample. The difference between the parental means is as obviously statistically significant, however, as in the previous year. The mean of the parental mean is not significantly different from the mean of the F_2 (difference = 0.09 ± 0.05) and the latter may be considered as intermediate between the two

Table 14

mm.										Variance	N				
2.50	2.75	3.0	3.25	3.5	3.75	4.0	4.25	4.5	4.75	5.0	5.25	5.5	5.75	Mean	
Old Irish	2.75	3.0	3.25	3.5	3.75	4.0	4.25	4.5	4.75	5.0	5.25	5.5	5.75	2.79±0.021	20
Long Eared	8	12	—	—	—	—	—	—	—	—	—	—	—	5.01±0.038	21
Nottingham	—	—	—	—	—	—	—	—	—	—	—	—	—	3.81±0.027	255
<i>F₁</i>	1	7	14	43	47	56	47	24	6	8	—	—	—	0.17465	

Table 15

(1)	Old Irish	Nottingham
(2)	Old Irish	Nottingham
(1)	Long Eared	Nottingham
(2)	Long Eared	Nottingham
(3)	Long Eared	Nottingham

parents. The range of the F_2 extends at the lower tail of the distribution to the full limit of the short rachilla parent, but values equal to the highest values of the long rachilla parent are not obtained at the upper tail of the distribution, the distribution stopping short at the lowest values of Long Eared Nottingham. There is no significant skewness in the distribution, the value for g_1 being $+0.104 \pm 0.152$. It may be stated, therefore, that the characteristics of the F_2 distribution and values bear out the straightforward intermediate value of the F_1 .

F_3 GENERATION

The F_1 and F_2 data did not suggest that any marked peculiarities in behaviour were being obtained in this study, and the problem of the study of the F_3 had to be considered in the light of this observation. The measurement of the whole of the F_3 was quite out of the question both from the point of view of the labour involved and the results likely to be obtained. Consequently, it was decided that F_2 plants from the upper and lower extremes of the distribution should be studied, and representatives of various length groups should be included as far as possible if the data thereby accumulated appeared to warrant it. Thirteen F_2 plants showing the longest rachilla values, and seven F_2 plants showing the shortest rachilla values, were chosen for special study, but 180 plants representing the various length groups in proportion to their occurrence in the F_2 were sown altogether. Forty-six grains were planted from each F_2 plant, but owing to the grain being three years old, and the growing season very dry, the germination, plant establishment and subsequent growth were very poor. This resulted in very few good F_3 plants being obtained and the F_3 investigation was nearly vitiated by these circumstances.

The data obtained from the twenty F_3 families chosen for special study are given in Tables 15 and 16. The distribution of rachilla lengths as given in Table 15 indicates the range exhibited by the families, but the small number of plants impairs the value of the results in some cases. However, the table does suggest the probable homozygous condition of families such as numbers 75, 97, 139, 145, 181 and 224, and the heterozygous condition of families such as numbers 41, 73, 96 and 193. The other families are more doubtful. In Table 16 the means of the F_3 families with their standard errors and variances are presented with the F_2 plant from which the family was derived. The variances, of course, give some indication of homozygosity, and the difference in these may

be compared in the families mentioned above. It may be observed that with four exceptions the rachilla length of the F_2 plant is less than the mean value of F_3 family derived from it. This is apparently due to the fact that the year in which the F_3 was grown was a "good rachilla year", because the values for the parents are also considerably greater. It is also interesting to notice that the short rachilla F_2 plants show by far the greater increase in the mean rachilla length of the derived family. The difficulty in assessing the behaviour of any F_2 plant in terms of the derived F_3 family grown under different conditions is emphasized by the data in Table 16, but such F_3 data may yet serve to disentangle the fluctuations from the genetic variances in the F_2 . For example, the value of the mean of plant 75 in the F_2 was 4.79 which is considerably shorter than the mean of Long Eared Nottingham in that year. But the mean of the derived F_3 family in the following year was 5.47 which is not significantly different from the value of Long Eared Nottingham in that year (Table 16), and the variance, though larger than the parent, is probably not significantly so. Similar conditions may be noticed in

Table 16

F_3 family no.	Rachilla length (in mm.) of F_2	parent plant	Mean	Variance	<i>N</i>
40	4.94		5.16 ± 0.086	0.10332	14
41	4.81		4.79 ± 0.096	0.12956	14
73	4.75		4.97 ± 0.093	0.14524	15
75	4.79		5.47 ± 0.076	0.05768	10
97	4.77		4.98 ± 0.040	0.01615	10
118	4.79		5.16 ± 0.148	0.10550	5
134	4.83		4.75 ± 0.070	0.02947	6
158	4.92		5.05 ± 0.102	0.11559	11
63	4.57		4.72 ± 0.202	0.16283	4
96	4.59		4.43 ± 0.319	0.40777	4
124	4.51		4.73 ± 0.086	0.09676	13
189	4.55		5.06 ± 0.109	0.10722	9
224	4.71		5.00 ± 0.095	0.07228	8
17	2.97		3.80 ± 0.259	0.26837	4
139	2.99		3.71 ± 0.086	0.11167	15
145	2.90		3.47 ± 0.115	0.08039	6
181	2.80		3.84 ± 0.076	0.06400	11
183	2.96		3.77 ± 0.095	0.11725	13
193	2.81		3.92 ± 0.182	0.27345	8
254	2.77		3.88 ± 0.122	0.07511	5
(1) Old Irish*	—		3.37 ± 0.071	0.08076	16
(2) Old Irish	—		3.28 ± 0.037	0.02235	16
(1) Long Eared Nottingham	—		5.58 ± 0.037	0.01509	11
(2) Long Eared Nottingham	—		5.54 ± 0.050	0.02766	11
(3) Long Eared Nottingham	—		5.59 ± 0.029	0.00950	11

* The parents were replicated in small beds throughout the F_3 material, and the figures given represent measurements made on different bed samples.

plant 145, whose mean was 2.90, while the mean of the derived F_3 family was 3.47 with a variance equal to that of the short rachilla parent Old Irish.

The study of these twenty extreme length types from the F_2 generation has done little to elucidate the genetic analysis. The parental types have apparently been recovered in a homozygous condition, and there appear to be heterozygous types in both the extreme long group and the extreme short group. There is, then, no suggestion of dominance in F_1 , F_2 or F_3 , and there is evidence that the factors are behaving in a simple additive way with no interaction.

DISCUSSION AND CONCLUSIONS

The investigations described in this paper, although in many ways incomplete, have shown that the length of the rachilla in barley is a heritable character of the quantitative type. Like all characters of this nature, it is very susceptible to the effects of environmental conditions, which influence all size characters in plants, presumably through the general metabolism. The rachilla is an inflorescence branch axis, and therefore like the length of the straw and the ear, and the internodes of both these parts, can be considered as a varietal character in spite of its environmental fluctuation. Owing to the fact that it is an inflorescence character, the length of the rachilla is not subject to the extreme fluctuations which operate on many vegetative characters, a fact which has been noticed in the many thousands of measurements made. For example, in the F_3 of Long Eared Nottingham \times Old Irish the plants were wretchedly grown and the grain very poorly filled and developed, but the rachillas of these plants were actually among the longest obtained in this cross. It is worth stressing what has been claimed in a previous account of rachilla length (Bell, 1937), that this character can be regarded as a useful addition to the limited number of grain characters which can be used in the identification and description of agricultural varieties of barley.

There appear to be a number of interesting facts about rachilla length in barley considered as a plant character. First, as will be mentioned below in discussing the genetics, there is a definite association between rachilla length and the type of hairs or bristles borne upon it. This was observed by Bell (1937) in a survey of two-row varieties which showed that in the "Chevallier" rachilla types the rachilla did not reach to the extreme shortness of the "Archer" rachilla types. Secondly, although the

rachilla has ceased to be a functional branch of the inflorescence, its length is still influenced by the length of the internodes of the main axis so that dense ear varieties with short internodes tend to have shorter rachillas than lax ear varieties with longer internodes. This association is seen also with the position on the ear, shorter rachillas arising from the upper portion of the ear where the rachis internodes become progressively shorter. It is interesting, however, that at the base of the ear, where the rachis internodes are usually somewhat shorter than at the position of maximum length a little higher on the ear, the rachillas are often relatively long, a condition which agrees with the general facts observed concerning lateral branches on an inflorescence. Finally, it has been observed that in six-row varieties of barley, where each spikelet of the triads borne at the nodes is fertile, the rachillas of the lateral spikelets are longer than that of the median spikelet of the same triad. This condition also is probably associated with the morphology of the system of branching in *Hordeum*, the lateral axes developing in a sympodial manner so that the second order of branches (lateral spikelets) are longer than the first order (median spikelets). Further, there is evidence that the difference in length between median and lateral rachillas varies in individual varieties.

The above considerations have to be borne in mind in the investigation and discussion of the mode of inheritance of rachilla length in barley, and the length character can only be studied when these possible complicating factors have been removed. When this is done, as in the series of crosses involving the same botanical types described in the second part of this paper, a straightforward quantitative genetic condition appears to be operative. The rachilla lengths of four of the five F_1 generations are intermediate in character, and two of the crosses show a small departure from the exact arithmetic mean of the parents. In the cross Long Eared Nottingham \times Old Irish, where the F_2 and F_3 were studied, there is no reason to suspect any anomalous condition. The F_2 of this cross gives a unimodal curve free from any skewness, and the mean does not deviate from the mean of the parents. It should be mentioned, however, that the longest fluctuants of the long rachilla parent were not obtained from the longest variants of the F_2 , although the shortest fluctuants of the long rachilla parent were represented. On the other hand, the short rachilla parent was recovered in the F_2 down to the extremely short fluctuants. The study of the F_3 was limited to twenty families obtained from plants selected from the tails of the F_2 distribution; indeed all the extreme types were studied. This limited study showed that homozygous F_3 lines

occurred with mean rachilla lengths equal to the parental means while obviously heterozygous lines also occurred at both ends of the distribution. In neither the F_2 nor the F_3 was there any suggestion of transgressive segregation, while the other four crosses of this series in which only the F_1 generation was studied showed no evidence of a heterotic effect, although in one of them there is a suggestion of dominance.

The conclusion to be drawn from these crosses, and particularly the Long Eared Nottingham \times Old Irish cross, is that when freed from the complicating interferences established previously, the length of the rachilla depends in inheritance on a system of multiple factors of a simple additive nature. There seems little need to postulate more than four major factors to explain the results.

In distinction to the results just described, the crosses between six-row varieties possessing different rachilla types present a different genetic scheme. In these crosses involving one "Archer" rachilla parent and one "Chevallier" rachilla parent the mean rachilla lengths of the F_1 generation (always of the "Archer" type) were at least as long as the long rachilla parent, and in some cases the F_1 mean exceeded that of the longer parent. This latter condition may, in the absence of complete analysis, be considered as a heterotic effect, but further study of this question is necessary. It is a matter of great interest that the dominance of the long rachilla appears to be independent of whether the "Archer" type or "Chevallier" type rachilla parent has the longer rachilla, because "Archer" type rachilla is dominant to "Chevallier" type, and varieties of the former type as a group are shorter than varieties of the latter type. In the cross *H. parallelum* \times Irish six-row, where the F_1 is longer than the longer rachilla parent, there is a marked transgression in the F_2 at the upper end of the distribution. On the other hand, in the cross *H. parallelum* \times Bigo the F_1 is equal to the rachilla of the longer parent and the F_2 shows a small transgression at both ends of the distribution. The means of the F_2 generations are consistent with the condition of the F_1 generation means in these two crosses.

Where the F_1 mean is longer than the mean of the long rachilla parent, the mean of the F_2 is equal to that of the long rachilla parent, but in the cross with the F_1 mean equal to the long rachilla parent, the mean of the F_2 is between the two parents but tending towards the longer one. From these observations there appears to be a form of dominance of the long rachilla in these two crosses.

The F_2 populations of both these crosses are most interesting as an example of a composite population composed of two quite distinct

constituent populations with their own statistical characteristics. Thus, when the unimodal F_2 composite populations are separated into the "Archer" and "Chevallier" types it is seen to form two curves in which the extracted "Archer" types cluster around a significantly lower mean than the extracted "Chevallier" types. Moreover, the means of the extracted types are significantly longer than the means of the corresponding parental rachilla types, with the exception of the "Chevallier" extracted types in the cross *H. parallellum* \times Bigo, where the increase in length does not reach significance. (It may be remembered that in this cross the F_1 was not significantly longer than the longer parent.) Another character of importance in these extracted populations is that the "Archer" type populations have a greater variance than the "Archer" type parent, whereas the variances are not significantly different between the extracted "Chevallier" type population and the "Chevallier" type parent. Because the "Chevallier" type rachilla is recessive to the "Archer" type, there appears to be a close linkage between the factors for rachilla length and rachilla type. There is some evidence to support the view that in these crosses, in which the extracted F_2 "Chevallier" population possesses as low a variance as the "Chevallier" type parent, there is one major factor for rachilla length, linked very closely with that of rachilla type, or alternatively, these factors may be identical. This view, which is based largely on the relative sizes of the variances, is not consistent, however, with the apparent heterotic effect in the F_1 and the "shift" of the means of the extracted F_2 populations above that of the parental means, unless a system of minor or modifying factors is also operative. It should be noted that the dominance of the long rachilla, with or without a heterotic effect, occurs in all crosses involving the "Chevallier" type rachilla (or its closely similar type seen in the form *Deficiens* II) and where the parents are either both of the six-row type, or of different fertility groups from the point of view of the development of the lateral spikelet. Although this dominance, with or without a heterotic effect, is not seen in the two-row \times two-row crosses, where both parents possess rachillas of the "Archer" type, it is quite possible that there is only a single major factor difference between the long and short rachilla forms studied in these crosses. It seems possible, therefore, that the evidence obtained from all the crosses described in this paper offers an example of the linkage between a qualitative and a quantitative character which may assist in future investigations in the inheritance of rachilla length.

SUMMARY

1. A study of the length of the rachilla in barley shows that it is a varietal character, although strongly affected by environmental factors.
2. Rachilla length as a varietal character is affected by the density of the ear and the type of hairs or bristles borne on the rachilla.
3. In six-row barleys the length of the rachilla of the lateral spikelet is greater than that of the corresponding median spikelet.
4. In the absence of any of the complicating factors mentioned above, rachilla length is inherited as a straightforward quantitative character with an intermediate F_1 and no signs of segregation in the F_2 . The number of major factors when the extremes of length are hybridized appears to be in the neighbourhood of four.
5. Hybridization of "Archer" and "Chevallier" type parents has given "dominance" of length and a possible heterotic effect. Long rachilla in these crosses appears to be linked with the "Chevallier" rachilla type.

The authors wish to express their indebtedness to Mr S. G. Stephens, Assistant Geneticist at the Cotton Research Station, Trinidad, for much of the data concerning the inheritance of the rachilla length in the six-row crosses. This data was obtained when Mr Stephens was working as a research student at the School of Agriculture, Cambridge.

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OBSERVATIONS ON THE MINERAL METABOLISM OF PULETS. V

ACID-BASE EQUILIBRIUM AND REPRODUCTIVE ACTIVITY

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(With Eight Text-figures)

SHELL secretion in the domestic fowl involves the metabolism of considerable amounts of fixed base. There is, therefore, a possibility that shell secretion may involve changes in the acid-base metabolism of the body and that such changes may be reflected in the plasma alkali reserve. The present experiments were carried out in order to see if the onset of reproductive activity in the fowl involves alterations in plasma alkali reserve.

Information with regard to the acid-base metabolism of the fowl appears to be limited. Burckhardt (1933) found that when lactic acid or hydrochloric acid were fed to the fowl in equivalent amounts, the lactic acid did not produce any signs of acidosis whereas the hydrochloric acid decreased the retention of nitrogen and phosphorus, increased the ammoniacal nitrogen of the urine and led to a negative calcium balance.

Solum & Schuster (1934) investigated the effects of various calcium salts upon the efficiency of a fattening ration, and concluded that the improvement of fattening results brought about by supplements of calcium carbonate or calcium lactate was due to their effect in preventing acidosis. The poor results secured with the basal ration alone or with a calcium chloride supplement were attributed to disturbances of acid-base metabolism.

Heller & Pursell (1937) have reported analyses of the fowl's blood throughout the life cycle; they were unable to detect significant changes in urea, non-protein nitrogen, creatinine, uric acid, glucose, sodium, cell chloride or plasma chloride during the first two years of life. However, if their data for red cell and plasma chloride be re-examined, it will be noted that in every case for which data are given, the ratio, percentage chloride in red blood cells : percentage chloride in plasma, is lower for laying birds than for non-laying birds. A lowering of this ratio represents a relative shift of chloride from cells to plasma; therefore the blood acid-

base equilibrium was relatively more alkalotic in the blood of the laying birds than in the blood of the non-laying birds.

So far as the immediate problem is concerned, the only significant direct observations on the blood acid-base equilibrium of the fowl appear to be those of Heller & Pursell, and these suggest that alkali reserve may be higher in normal laying birds than in similar non-laying birds. Balance experiments (Common, 1932) have shown that pullets tend to display an increasing retention of calcium over a period of about two weeks or so before laying begins; in many cases, especially where the ration is high in calcium carbonate, this is accompanied by a remarkably high Ca/P retention ratio (Common, 1936). These facts suggest that the mineral which is being deposited in the skeleton at such times may have a Ca/P ratio higher than that of the skeleton as a whole. It may be remarked that the carbonate content and hence the Ca/P ratio of the skeleton in various species appears to be related to the blood alkali reserve for the species in question, low alkali reserve being associated with low carbonate content (Morgulis, 1931).

On low calcium rations the skeleton is rapidly depleted of calcium for shell formation with a concomitant excretion of phosphorus (Common, 1932), and where depletion has proceeded for some time the composition of the skeleton is altered in the sense that the bone mineral has a lowered carbonate content and a lowered Ca/P ratio (Common, 1938). This at first suggests a physiological acidosis, for it is well known that acidosis induced by mineral acids reduces the carbonate of bone mineral and may even remove calcium from the skeleton without removing significant amounts of phosphate (Goto, 1918). On the other hand, Tyler (1940) has recently adduced evidence that the laying fowl, even when receiving adequate amounts of calcium carbonate, removes calcium intermittently from the skeleton for shell formation without removing phosphate in significant amounts. Tyler points out that these intermittent drafts on bone calcium appear to be determined by the fact that the capacity of the fowl to retain calcium from the food does not greatly exceed 1 g. per diem, whereas the calcium in an average egg shell is considerably greater than this. The question thus arises as to whether or no the blood acid-base equilibrium of laying birds receiving a diet adequate in calcium is shifted towards the acid side. The data of Heller & Pursell (1937) give no grounds for an affirmative answer. Moreover, in the case of actively laying birds receiving diets adequate in calcium, and where there must be a recurrent mobilization of skeletal calcium alternating with replenishment of this depot, there does not appear to be any pronounced decrease in skeletal

Ca/P ratio provided that a heavy total draft on skeletal calcium does not take place (Common, 1938).

EXPERIMENTAL

A group of four White Wyandotte pullets (pullets N 1 to N 4) was fed on the basal ration and determinations of plasma alkali reserve were carried out from time to time. A similar group (pullets K 1 to K 4) was treated in the same way except that the basal ration was supplemented with 5% calcium carbonate. The calcium and phosphorus balances were determined on a basis of 5-day periods. The apparent digestibility of the phytic acid phosphorus in the rations was also determined in order to amplify some previous observations on this point.

Calcium was determined volumetrically (Godden, 1937) and phosphorus by the method of Fiske & Subbarow (1925); magnesium was determined gravimetrically as magnesium ammonium phosphate after removal of calcium. In the case of bone analyses phosphorus was determined gravimetrically. Carbonate determinations were made on the powdered bone by the Collins calcimeter using 1 g. samples. Phytic acid phosphorus was determined by a modification (Common, 1940a) of the method of McCance & Widdowson (1935). Plasma alkali reserve was determined on oxalated plasma by the Cullen-Van Slyke method using the manometric Van Slyke apparatus (Peters & Van Slyke, 1931).

No difficulty was experienced through the repeated withdrawal of blood samples, the birds' food consumption and egg production being apparently unaffected, but it was noticed that the clotting power of the blood was decreased in the case of the later bleedings of K 2, K 3 and K 4. This suggests that the ration was deficient in vitamin K for heavily laying birds, and that vitamin K is either a food constituent normally laid down in the egg or a factor used up by the process of egg formation.

The birds were killed at the end of their balance periods and their dry fat-free tibiae analysed for calcium, phosphorus, magnesium and carbon dioxide.

The rations were made up as follows:

	Ration N lb.	Ration K lb.
Yellow maize meal	40	40
Middlings	30	30
Sussex ground oats	20	20
Extracted soya-bean meal	7.5	7.5
Fish meal	2.5	2.5
Cod-liver oil	2.0	2.0
Salt	0.5	0.5
Calcium carbonate	5.0	—

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The cod-liver oil was guaranteed to contain not less than 100 i.u. vitamin D and 1000 i.u. vitamin A per g.

The composition of the rations are given in Table 1.

Table 1. *Analytical data for rations*

	Ration N (low calcium)		Ration K (high calcium)	
	%	Coefficient of variation	%	Coefficient of variation
Ca	0.231	3.07	2.171	1.59
P	0.561	2.26	0.538	2.64
Mg	0.194	3.33	0.193	0.68
Phytic acid P	0.324	2.46	0.321	3.80

The low calcium ration N contained 0.231% calcium on account of the small proportion of fish meal included. If the ration contains still lower amounts of calcium, as in the case of unsupplemented cereal rations, it may not be easy to bring the birds into lay and egg eating is almost certain to ensue. For the purposes of the present experiment a content of 0.231% calcium was sufficiently low, but not so low as to introduce these difficulties on an undue scale.

The live weights of the pullets during the experiment are tabulated in Table 2, from which it will be seen that in general live weights were satisfactorily maintained even in the case of heavily laying birds (pullets K 3 and K 4). Pullet N 3 lost in eight somewhat towards the end of the experiment; this bird's appetite and food consumption were rather low, especially in the latter stages of the experiment when her laying activity was over.

Table 2. *Live weights of experimental birds*

Day of experiment	Live weight in kg.						
	N 1	N 2	N 3	K 1	K 2	K 3	K 4
0	1.73	1.68	1.54	1.74	1.74	1.66	2.14
11	1.78	1.98	1.81	1.96	1.89	1.81	2.21
25	1.96	2.23	1.98	2.10	2.05	1.75	2.30
39	2.01	2.28	2.09	2.13	2.17	1.85	2.35
53	2.05	2.31	1.94	2.22	2.31	1.94	2.49
67	2.11	2.39	1.91	2.15	2.39	2.04	2.58
81	2.07	2.40	2.03	2.19	—	2.07	2.46
95	2.06	2.46	1.87	2.12	—	2.06	2.38
109	—	2.43	1.85	2.17	—	1.97	—
123	—	—	—	2.14	—	2.09	—

EXPERIMENTAL RESULTS

The main experimental results are set out in graphical form in Figs. 1-8. In these figures the calcium balance denotes the total calcium balance for the 5-day period in question and includes the total egg calcium. The eggs are indicated and reckoned into the balance for the day preceding oviposition, since most of the eggs were laid in the early morning or forenoon.

The upper curves in each case indicate the plasma alkali reserve in vol. CO_2 % of plasma (A.R.).

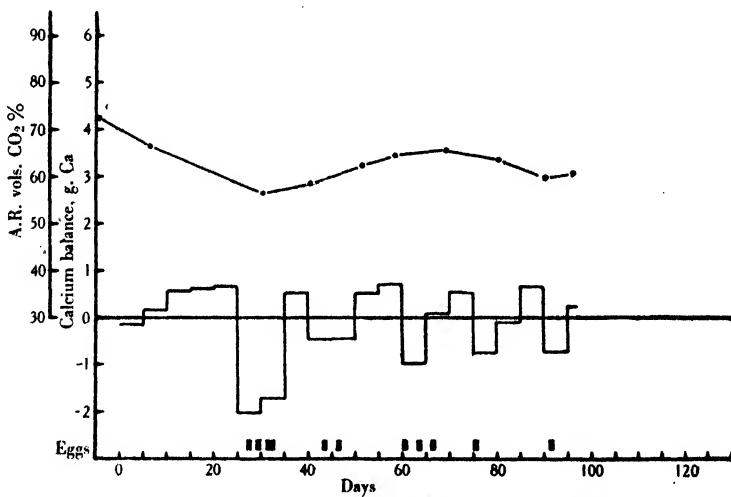


Fig. 1. Pullet N 1. Low calcium ration. 11 eggs laid.

Figs. 1-3 relate to pullets N 1, N 2 and N 3. Pullet N 4 was a persistent egg eater and was killed after a relatively short period; hence the data for this bird are of little value so far as plasma alkali reserve is concerned and are therefore omitted. Figs. 4-7 relate to pullets K 1, K 2, K 3 and K 4 and Fig. 8 relates to a cock.

The first alkali reserve determinations on the curves relate to the first morning on which the experimental rations were fed; the balances were begun 5 days later.

The first striking point about the results is the contrast between the A.R. curves for the pullets receiving the low calcium ration N and those receiving the high calcium ration K. In the case of the birds receiving ration N the A.R. drops slightly at the outset and then remains at a level

in the region of 60 vol. $\text{CO}_2\%$. Pullets N 1 and N 2 display a slight tendency to return to higher levels later on, but there is no clear-cut

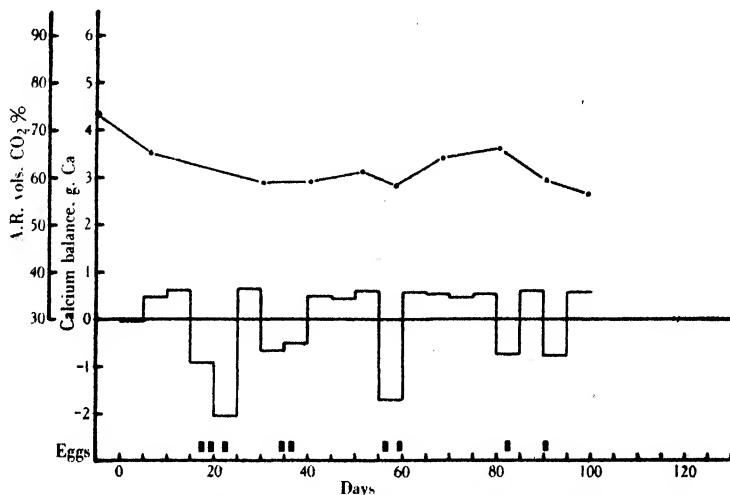


Fig. 2. Pullet N 2. Low calcium ration. 9 eggs laid.

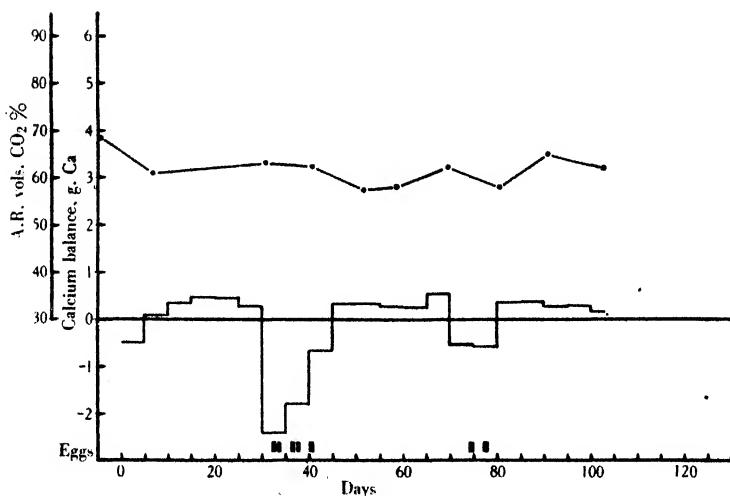


Fig. 3. Pullet N 3. Low calcium ration. 7 eggs laid.

relationship between A.R. and calcium balance in any of these birds. On referring to Fig. 8 it will be noted that the A.R. for the cock followed a very similar course while receiving ration N.

On turning to the results with the pullets receiving the high calcium ration K, it will be seen that in the cases of pullets K 2, K 3 and K 4 (Figs. 5-7) the A.R. rises to over 80 vol. $\text{CO}_2\%$ during the pre-laying period, which was in each case also a period of increasing calcium storage. During the laying periods there is an obvious tendency for the A.R. to drop somewhat, and in general the A.R. tends to run parallel with the total calcium balance. (The anomalous data for K 3 during the first 15 days are due to the very capricious appetite of this bird over this period.) The pre-laying increase in A.R. and the maintenance of a high level of the A.R. during laying displayed by the pullets on the high calcium ration K constitute the main results of the present experiment.

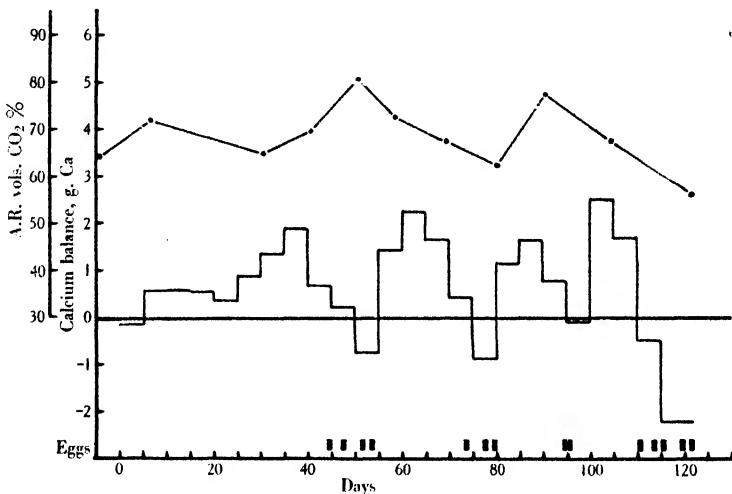


Fig. 4. Pullet K 1. High calcium ration. 14 eggs laid.

It may be argued that these results are purely an effect of the high calcium intake and that reproductive activity is not necessarily involved. This argument does not account for the fact that the A.R. of the cock (Fig. 8) displayed no similar increase to high levels of A.R. when placed on ration K; it is therefore more probable that one of the changes in the physiology of the fowl brought about by the onset of reproductive activity is a displacement of the acid-base equilibrium of the body so that the A.R. will rise to a high level, provided that the food supplies adequate amounts of base for this effect to manifest itself.

At first sight the results with K 1 (Fig. 4) do not appear to accord with the results for pullets K 2, K 3 and K 4. However, it is noteworthy that this bird displays a very poor capacity for calcium retention in spite of the

fact that food consumption, and hence calcium intake, was satisfactory and steady. The tendency for A.R. to increase during the pre-laying

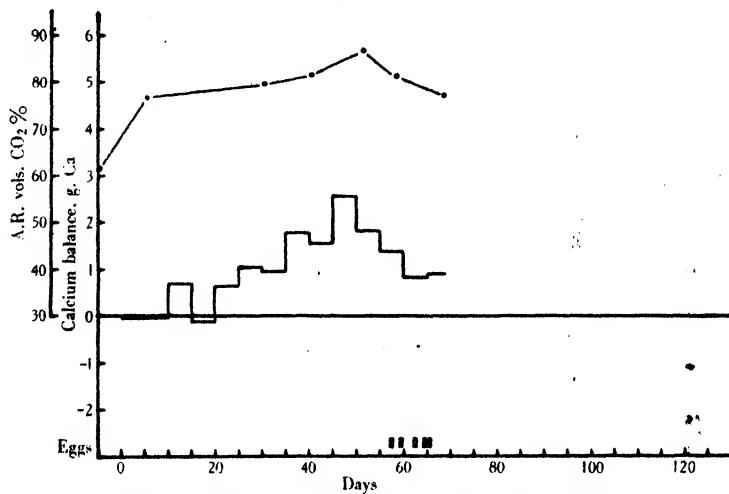


Fig. 5. Pullet K 2. High calcium ration. 5 eggs laid.

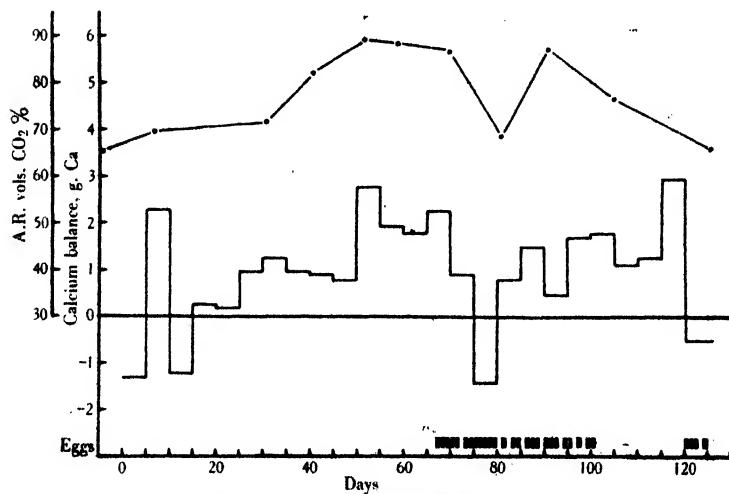


Fig. 6. Pullet K 3. High calcium ration. 30 eggs laid.

period is less pronounced than in the cases of K 2, K 3 and K 4; this is probably related to the lower pre-laying storage of calcium. During the laying period the calcium balance of pullet K 1 is frequently negative in

spite of the low intensity of laying, and the A.R., which never rises as high as in the other pullets of group K, is not so clearly related to the fluctua-

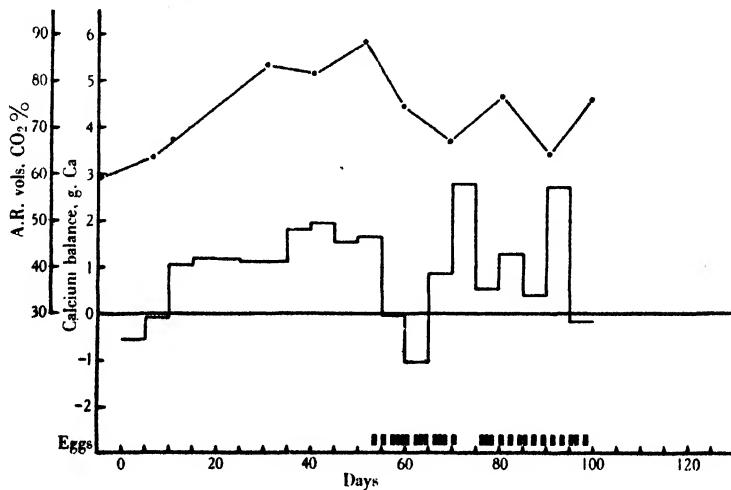


Fig. 7. Pullet K 4. High calcium ration. 27 eggs laid.

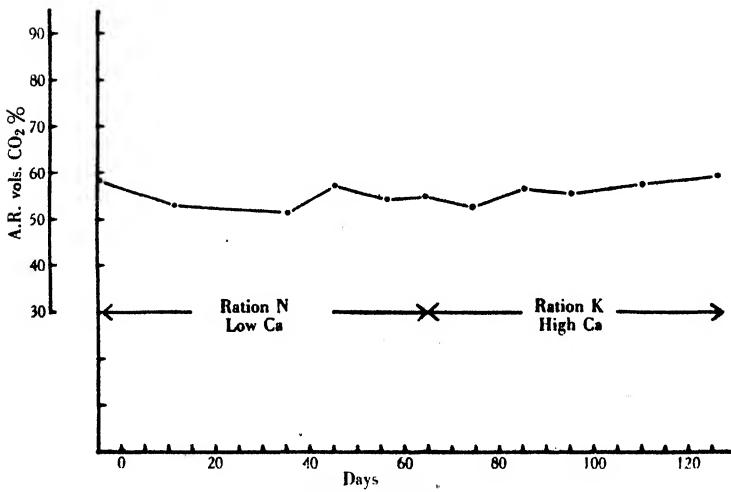


Fig. 8. Cook:

tions in calcium balance. There is, in fact, a general difference in both A.R. and calcium metabolism as between pullet K 1 and the other three pullets of group K. The very divergencies between the results for K 1 and

the other pullets of group K are such as to strengthen the view that the A.R. is affected by reproductive activity as well as by calcium carbonate intake.

Taken as a whole the present observations substantiate the suggestion implicit in the data of Heller & Pursell (1937) that laying birds tend to have a higher blood alkali reserve than non-laying birds when the ration is not deficient in calcium.

The calcium retention and Ca/P retention ratios for periods preceding the first laying period are given in Table 3.

Table 3. *Pre-laying calcium retention and Ca/P retention ratios*

Pullet	Period before first laying period								Total for pre-laying periods	
	4th		3rd		2nd		1st		Ca	Ca/P
	Ca	Ca/P	Ca	Ca/P	Ca	Ca/P	Ca	Ca/P		
N 1	0.18	0.53	0.56	1.47	0.62	1.51	0.67	1.29	2.03	1.23
N 2	—	—	0.04	0.22	0.44	1.13	0.60	1.54	1.00	1.28
N 3	0.34	0.92	0.46	1.15	0.43	1.05	0.29	0.41	1.52	0.81
N 4	—	—	—	—	0.04	0.14	0.39	0.95	0.43	0.61
K 1	0.36	1.24	0.88	2.10	1.34	2.53	1.89	2.96	4.47	2.38
K 2	1.79	2.56	1.55	2.42	2.55	3.98	1.81	3.07	7.70	2.99
K 3	0.76	1.73	2.75	3.20	1.92	3.43	1.79	2.49	7.22	2.80
K 4	1.12	2.61	1.80	2.77	1.95	2.75	1.53	2.51	6.40	2.67

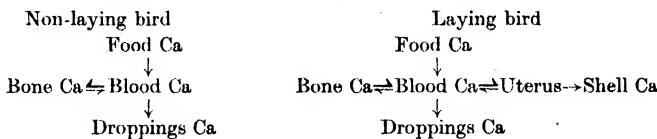
Higher Ca/P retention ratios prevail in group K than in group N. Among the birds of group K, pullet K 1 is distinguished by a tendency to a lower Ca/P retention ratio than the other three birds; as pointed out above, this bird also displayed a poorer capacity for calcium metabolism and maintained a lower A.R. level than the other birds of group K.

DISCUSSION OF RESULTS

As the laying period approaches pullets receiving a diet adequate in calcium carbonate begin to store calcium at an increased rate; this calcium must be stored in forms of higher Ca/P ratio than the average Ca/P ratio for normal bone, and the present observations strongly suggest that the period of increasing storage is also a period during which the blood A.R. is increasing. When laying begins, blood A.R. may fall somewhat as the total calcium balance decreases due to egg laying, but consideration of the course of blood A.R. during the laying period introduces some difficulties.

Even in the case of birds on high calcium rations the skeleton is drawn upon for calcium during shell formation because the capacity of the bird to retain calcium from the food is limited. Tyler (1940) has

brought forward evidence in support of the view that the calcium compounds thus mobilized from the skeleton have a very high Ca/P ratio; and there is evidence that appreciable amounts of calcium may be withdrawn without any concomitant withdrawal of phosphate provided the diet is adequate in respect to calcium. The question therefore arises as to how a high alkali reserve in the laying bird, associated with deposition of bone mineral of high Ca/P ratio, can be reconciled with rapid intermittent drafts on the skeleton which remove a bone mineral fraction also of high Ca/P ratio, an effect which at first sight suggests an acidosis. It is known that the immediate stimulus for shell secretion is mechanical (Tarchanoff, 1884; Pearl & Surface, 1909), but there is no evidence as yet that the stimulus to secretion involves intermittent periods of acidosis to mobilize bone mineral of high Ca/P ratio alternating with periods of relative alkalosis to permit of replenishment of the same bone mineral components. The available information suggests that during the laying period a bird receiving adequate calcium carbonate maintains an increased blood A.R. and that when calcium is being deposited in the skeleton the material laid down has a higher Ca/P ratio than bone, and may even consist mainly of carbonate. When the mechanism of shell secretion is called into operation from time to time, rapidity of calcium carbonate deposition on the shell exceeds the rapidity with which calcium carbonate and basic calcium phosphates can be laid down in the skeleton. The equilibrium is displaced so that not only is the absorbed calcium deflected from deposition in the bones to shell formation, but also so that bone mineral of high Ca/P ratio previously laid down in the bones is drawn upon for shell formation. Immediately shell secretion stops, replenishment of bone carbonate, or of bone mineral of high Ca/P ratio, begins anew. In other words, so long as the shell gland is active, its potential of calcium excretion exceeds the potential of calcium deposition in the skeleton. This may be indicated diagrammatically:



The mechanism governing calcium mobilization for shell secretion is obscure. Knowles *et al.* (1935) suggested that the parathyroids were chiefly responsible, but it would appear from more recent work (Avery *et al.* 1940a, 1940b) that there is some lack of agreement as to the precise action of parathyroid hormone on the fowl. Emphasis appears to be

shifting from the parathyroids to the ovary as the regulator of mobilization of shell calcium and Deobald *et al.* (1938) suggest that oestrin is one of the factors responsible for the observed blood calcium and blood phosphorus variations in the laying pullet. The present experiments suggest that acid-base regulatory mechanisms are also implicated in the changes which take place in the blood of the laying fowl. It is clear, however, that any theory of the mechanism governing this mobilization of bone calcium for shell formation must also explain the effects of such drafts upon the composition of the bone mineral and the differential removal of a fraction of bone mineral of high Ca/P ratio.

Further evidence of differential effects of calcium storage and mobilization upon the composition of the bone mineral of the pullet was secured in the present experiment by analysing the dry fat-free tibiae of the birds at the conclusion of the experiment. The results in general confirmed previous work on this point, but it was observed that the tibiae of the pullets of group K, all of which displayed a positive cumulative calcium balance during their laying period, had a remarkably high ratio carbonate Ca : total Ca. The ratios were 0.153, 0.150, 0.134 and 0.153 for pullets K 1, K 2, K 3 and K 4 respectively. This suggests that a laying pullet may maintain or even increase the proportion of carbonate calcium in her skeleton despite the recurrent drafts on the residual calcium of the bone during laying, provided, of course, that the cumulative calcium balance is positive. Where the cumulative balance is negative this does not necessarily hold. It is clear that the composition of the bone mineral in the pullet is subject to relatively wide variation according to diet and calcium balance.

The apparent digestibility of phytic acid phosphorus

Previous experiments (Common, 1940b) provided some indication that the apparent digestibility of phytic acid phosphorus, which is greatly reduced by supplements of calcium carbonate, might be increased again slightly during laying by reason of the removal of calcium from the gut for shell formation. It was felt that the present experiment might provide some further evidence on this point, and the relevant data are set out in Table 4. It may be pointed out that such an effect of laying on the digestibility of phytic acid phosphorus was not anticipated in the case of group N, for here the food calcium was very low relative to the food magnesium. However, the apparent digestibility in the case of group N did display a steady tendency to increase during the course of the experiment, although the effects could not be clearly related to egg formation.

Of the birds in group K, pullet K 1 laid at a very low intensity and K 2 was killed after a very short laying period. In neither case was there evidence that laying increased the apparent digestibility of phytic acid phosphorus.

Table 4. *Apparent percentage digestibility of phytic acid phosphorus*

Ration N contained 0.324% phytic acid P and ration K 0.321%.

Pullet no.	N 1	N 2	N 3	K 1	K 2	K 3	K 4
Entire experiment	64.4 (97)	53.6 (100)	61.6 (103)	15.9 (122)	13.0 (69)	19.8 (126)	18.2 (100)
Pre-laying periods	55.1 (25)	40.9 (15)	51.5 (30)	13.1 (40)	14.1 (55)	15.5 (65)	11.4 (50)
Laying periods	67.7 (40)	52.9 (35)	67.7 (25)	17.0 (47)	8.6 (14)	21.5 (48)	25.0 (50)
Non-laying periods excepting pre-laying periods	69.9 (62)	59.1 (65)	68.2 (78)	18.7 (75)	—	13.5 (15)	—

The figures in brackets indicate the number of days involved.

In the case of pullet K 3, and still more in the case of pullet K 4, the apparent digestibility was definitely higher during the laying periods than during non-laying periods.

The results suggest that an increase of apparent digestibility of phytic acid phosphorus due to the calcium metabolism of shell formation will only become pronounced when birds on a moderately high calcium carbonate intake begin laying intensely. On low calcium rations the effect may not be evident.

SUMMARY

1. Calcium and phosphorus balance experiments with pullets during which the plasma alkali reserve of the birds was followed are described. Evidence is produced in support of the view that the onset of reproductive activity in the pullet is accompanied by an increase in plasma alkali reserve, as well as of calcium retention, provided the ration contains adequate calcium carbonate.

2. These high levels of plasma alkali reserve tend to decrease with the beginning of laying, but apparently the plasma alkali reserve of laying birds tends in general to remain higher than that of the non-laying bird or of cocks.

3. The possible bearing of these observations on bone mineral composition and Ca/P retention ratios is discussed.

4. It is shown that on a cereal ration moderately low in calcium, where the apparent digestibility of phytic acid phosphorus is of the order of 40-70%, it was not possible to correlate apparent digestibility with the

calcium metabolism of shell formation. When the same ration was supplemented with 5% calcium carbonate, so that the apparent digestibility was of the order of 9-25%, then it was possible under circumstances of intense laying to detect a slight increase of apparent digestibility in association with laying.

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THE PLACEMENT OF FERTILIZERS

I. ROOT CROPS

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INTRODUCTION

IT is common sense that fertilizers should be applied when and where they can be most effectively used by crops, but, although *when* to apply fertilizers has been the subject of many experiments, the problem of *where* to apply them has not received the attention it deserves. Until recently it has been widely assumed that uniform distribution of fertilizer over the surface of the soil is enough, yet brief consideration of the behaviour in soil of the three major nutrients reveals grounds for doubt.

Nitrogen in the form of nitrate is mobile in soils, the movement being mostly downwards, so that care is needed to ensure that added nitrogen is not leached out of range of the plant roots. Uniform distribution over the soil surface is usually assumed to be particularly desirable for nitrogen fertilizers, but this assumption has been challenged by Wright (1938), who suggested that the importance of even distribution may be exaggerated. Trials conducted by the staff of the Institute for Research in Agricultural Engineering of Oxford (*Agricultural Engineering Record*, 1940) have shown that very uneven top dressing of wheat with nitrochalk gave just as good yields of grain and straw as very even application.

When water-soluble phosphate is incorporated in soil, it is rapidly and firmly fixed—so firmly in some soils as to be almost unavailable to crops. There is no surer means of fixing phosphate than broadcasting followed by harrowing. Phosphate is needed particularly in the early stages of plant growth, and it seems that the needs of many crops would be best met by applying phosphate in a restricted zone near the seed.

Potash behaves somewhat like phosphate in the soil and is fixed so that it remains more or less where it is applied. If potash is not so firmly fixed as to be unavailable, and this seldom happens in British soils, there is no apparent reason why potash should not be applied to arable crops at the same time and in the same way as phosphate.

Restricted application in a zone near the seed of the phosphate and potash is, however, not necessarily ideal. Indeed, as Garner (1938) has

pointed out, the root range of most crops is far greater than 9 in. and includes the subsoil, which is nearly always poor in nutrients, so that incorporating fertilizers near the surface may not give the best results. Experiments by the Rothamsted workers suggest that for sugar beet it may be better to incorporate phosphate and potash at some considerable depth in the soil.

At the opposite end of the scale to broadcasting is applying fertilizers in the drills in contact with the seed. This is practised with considerable success in certain instances, but it involves risk of reduced germination and harm to seedlings. On the whole, phosphate is least harmful and potash most harmful when applied in this way; the effect of nitrogen is intermediate. The risk of damage also varies greatly with the crop; root crops, except potatoes, are highly susceptible whereas sowing fertilizers in the drills with cereal seed causes only a slight delay in braiding and does not affect the final stand of plant. Because of this difference, root crops and cereals are best dealt with in separate papers.

This paper deals with the problem as it applies to root crops. In the first section, experimental evidence is given to show that broadcasting is not the best method of applying fertilizers; in the second section, the results of experiments in which fertilizers were applied in several ways are presented. The potato crop is not considered since many experiments have shown that concentrating dung and fertilizers in the ridges gives higher yields and a bigger percentage of ware tubers than broadcasting.

EVIDENCE OF ADVANTAGES OF RESTRICTED APPLICATION OF FERTILIZERS

Swedes, turnips and kale

It is widely assumed that if soluble fertilizers are drilled in contact with the seed of swedes, turnips or kale, germination is impaired and many young seedlings are killed. The writer has seen fields where hardly a seedling appeared when swedes and kale were drilled in contact with a complete fertilizer. In certain parts of England, superphosphate is, however, often drilled with swede and kale seed and good crops result. In the areas concerned this practice facilitates early horse-hoeing, as the rows are more clearly defined and weed growth is much less than when superphosphate is broadcast.

The results of four experiments, conducted in 1935 in Wiltshire and Dorset, where superphosphate is often sown in the drills, are given in Table 1. At centre 1, phosphate had no effect when broadcast but sig-

Table 1. *Sweden experiments in Wiltshire and Dorset*

		(1) Salisbury		(2) Wimborne		(3) Blandford		(4) Dorchester	
		Tons per acre	No. of roots per acre	Tons per acre	No. of roots per acre	Tons per acre	No. of roots per acre	Tons per acre	No. of roots per acre
1	No fertilizer	7.2	14,800	2.9	20,000	11.0	17,000	4.6	20,600
	P	7.2	14,200	5.1	21,100	12.0	17,900	4.2	22,200
2	P	7.5	15,100	4.8	20,600	11.1	16,800	5.4	20,400
	PK	8.6	15,500	6.8	20,800	11.0	17,500	5.6	23,100
3	NPK	10.5	19,600	5.7	20,200	12.4	18,200	5.2	23,200
	P	8.4	17,100	5.0	19,900	10.7	15,000	2.6	12,800
4	PK	7.4	14,800	8.0	21,100	11.4	15,600	3.9	17,500
	NPK	2.9	5,200	1.1	2,100	3.8	2,600	1.8	6,200
Significant difference ($P=0.05$)		2.5	4,500	1.0	1,800	3.3	2,300	1.5	5,400
Comparison of 2-7									
Significant difference ($P=0.05$)									
Comparison of 2-7 with 1									

P=4 cwt. superphosphate (14% P_2O_5) per acre. N=1 cwt. nitrochalk per acre. K=1 cwt. potash salts (30% K_2O) per acre.

nificantly increased the yield when sown in the drills. At centres 2, 3 and 4 there was no significant difference between the two methods of application. Only at centre 2 was there a response to phosphate. Potash salts had no effect when broadcast, but reduced the yield and stand of plant at three centres when sown in the drills. Nitrochalk significantly increased yields at centre 2 and did not do harm at any centre when sown in the drills.

These results confirm local opinion that superphosphate is generally as effective and sometimes more effective when sown in the drills than when broadcast, that potash in the drills is harmful, and that nitrogen in the drills is no more effective than when broadcast. It must be emphasized that these experiments were conducted in a particular area where the soils generally are very deficient in phosphate, and it must not be assumed that sowing superphosphate in the drills with swede seed always leads to success. Experiments in New Zealand (Tennant, 1930) have shown that putting superphosphate in the drills may reduce the stand of plant and give lower yields than broadcasting. Results of a braiding test given in Table 2 confirm that superphosphate may cause harm when sown in the drills.

Table 2. *Swedes: percentage germination*

Days after sowing...	7	8	9	10	11	12	18	24
O	26	57	70	74	76	81	83	85
N	3	12	24	29	32	35	42	44
P	3	11	18	24	25	28	33	32
K	4	14	19	22	26	31	44	54
NP	1	6	12	15	17	17	19	21
NK	0	1	3	4	6	6	18	24
PK	1	8	11	12	12	12	15	18
NPK	1	4	6	11	12	12	15	18

N = 1 cwt. sulphate of ammonia per acre. P = 5 cwt. superphosphate per acre.

K = 1 cwt. potash salts per acre.

A clue to the cause of the variable effect of superphosphate is given by sugar-beet experiments reported below and appears to lie in the soil; on soils markedly deficient in phosphate, sowing superphosphate in the drills is very effective but on soils containing a moderate supply of available phosphate it is harmful.

MANGOLDS AND SUGAR BEET

In Devon and Cornwall, mangold seed is often sown with a canister drill, and a complete fertilizer at a heavy rate is applied along the drills which are about 3 in. wide. Strange as it may seem, the practice is

reasonably successful. The results of a number of experiments summarized in Table 3, show, however, that at about one centre in four, the yield was not increased. In every case failure to increase yields was associated with a reduction in plant numbers. The results of two experiments conducted in 1934 to compare broadcasting the fertilizer and sowing it in the drills are given in Table 4. At centre 1, sowing fertilizer in the drills gave a significantly higher yield of roots than broadcasting, despite a reduced stand of plant. At centre 2, sowing fertilizer in the drills gave a much lower yield than broadcasting.

Table 3. *Mangolds: summary of significant effects of fertilizer**
Numbers of experiments

	Yields				No. of roots		
	Total	Increase	Decrease	No change	Increase	Decrease	No change
1932, 1933	4	4	0	0	2	0	2
1934	6	2	1	3	1	4	1
1935	7	7	0	0	2	0	5
1936	5	3	0	2	1	2	2
Total	22	16	1	5	6	6	10

* 404 lb. sulphate of ammonia, 640 lb. superphosphate, 98 lb. sulphate of potash and 91 lb. muriate of potash per acre.

Table 4. *Mangolds: broadcasting v. placement in drills*

	Centre 1 Manaccan		Centre 2 Bovey Tracey	
	Tons per acre	No. of roots per acre	Tons per acre	No. of roots per acre
No fertilizer	19.8	19,300	22.0	17,100
Fertilizer* broadcast	33.4	21,300	24.9	15,200
Fertilizer* in drills	37.9	19,900	19.8	12,000
Significant difference (P = 0.05)	3.5	1,400	2.7	1,900

* Means for five treatments each supplying 83 lb. N, 83 lb. P₂O₅ and 100 lb. K₂O per acre.

Very few experiments appear to have been conducted in this country on methods of placing fertilizers for sugar beet. McMillan & Hanley (1935) found in three experiments that 4 cwt. per acre of a complete fertilizer, sown in the drills, slowed up germination and gave a rather smaller final plant population than broadcasting. In braiding tests, they found that 1 cwt. superphosphate per acre in the drills had little effect, but 3 cwt. was slightly harmful. Similarly 1 cwt. sulphate of ammonia had little effect, but 2 cwt. seriously retarded germination. Potash salts

at 1 and 2 cwt. per acre was about as harmful as 2 cwt. sulphate of ammonia. The experiments were made under dry conditions.

The results of braiding tests with sugar beet on two soils, one very deficient in phosphate and the other very deficient in potash, are given in Table 5. They afford an important clue to the variable effects, described above for other crops, of sowing fertilizers in the drills. Sowing superphosphate in the drills improved the rate of braiding on the phosphate-deficient soil and sowing potash in the drills improved the rate of braiding and the final stand of plant on the potash-deficient soil.

It appears, therefore, that where any one nutrient is markedly deficient, its application in the drills in contact with the seed may improve braiding and the stand of plant, and increase yields more than when it is applied broadcast. Nevertheless, sowing nutrients in the drills is a potential source of harm which is manifest on soils not very deficient in any nutrient. Application of fertilizers in a restricted zone near, but not in contact with the seed, might, therefore, be expected to give better results than broadcasting.

Table 5. *Sugar beet: number of seedlings per 4 yard rows*

Days after sowing ...	(120 'seeds' sown)						Potash-deficient soil				
	Phosphate-deficient soil										
	6	7	8	10	12	19	8	9	10	13	19
O	40	80	115	163	185	188	85	148	156	174	174
N	1	5	14	75	130	175	4	66	112	174	184
P	73	118	149	191	197	198	55	98	127	157	160
K	3	11	32	76	100	149	52	154	191	219	222
NP	8	36	69	120	142	159	1	47	102	171	176
NK	0	2	15	54	80	112	2	19	64	166	191
PK	22	65	111	169	181	182	8	60	117	183	191
NPK	29	74	116	163	176	183	5	58	105	137	163
Significant difference ($P=0.05$)				48	40	64		37	32	35	

N = 42 lb. N per acre. P = 42 lb. P_2O_5 per acre. K = 50 lb. K_2O per acre.

EXPERIMENTS ON VARIOUS METHODS OF PLACING FERTILIZERS

In recent years a large number of experiments has been conducted in the U.S.A. on the placement of fertilizers. Judging by the literature (*Proceedings*, 1936-9), the practice has been reduced to a fine art. The American experiments show that no one method of placement is universally the best. Placing fertilizer in bands at the sides of the seed has generally given the most favourable results, but in some cases sowing fertilizer below the seed or in contact with the seed has proved better.

The most suitable distance of the fertilizer bands from the seed seems to vary for any one crop; fluctuations in rainfall and differences in soil appear to account for these variations.

Ideally, the placement of nitrogen, phosphate and potash should be considered separately, and a start has been made on this in America. As in most of the American work, the placement of complete (NPK) fertilizers is the subject of the experiments described below. A description of the drill used to sow the seed and fertilizer is given in the Appendix.

The results of seven experiments on swedes are given in Table 6. In the first experiment, the coulters used to sow the fertilizer in bands at both sides of the seed, were 4·5 in. apart (2·25 in. from the seed) and were staggered in the hope of preventing blockage by clods of soil. Although the seed bed was fairly fine some blockages occurred. In this experiment there were no significant differences in yield, but sowing the fertilizer below the seed reduced plant numbers. This reduction was not due to fertilizer coming in contact with the seed at sowing time; the seed bed was reasonably deep so that the coulters did not 'ride' and chains were fixed behind the fertilizer coulters to ensure covering the fertilizer with soil.

It was the opinion of the writer that in Exp. 1, the fertilizer bands were too far from the seed, so in Exps. 2-7, the bands were sown 1½ in. from the seed and the coulters were staggered more than in Exp. 1; the results are given in column A. The makers of the drill suggested obviating the need to stagger the coulters by setting them wider apart. Although it appeared that the fertilizer would be too far from the seed, the arrangement was tried; the results are given in column B.

In five out of six experiments where fertilizers increased yields, broadcasting gave lower yields than one or other of the methods of restricted placement. In Exp. 6, fertilizer in bands 3½ in. from the seed gave higher yields than in bands 1½ in. from the seed; this was due to clods jamming between the closer coulters and causing bad sowing of seed and fertilizer with a resultant poor stand of plant. In Exp. 7, where the seed bed was good and free from clods, fertilizer in bands 1½ in. from the seed gave higher yields than in bands 3½ in. from the seed. In Exps. 1 and 2, fertilizer below the seed gave poorer results than when in bands 1½ in. from the seed, whereas in Exps. 4 and 7 the former gave better results. These differences were probably due to the soil; at centres 4 and 7, the soil was known to be very deficient in phosphate whereas the soil at centres 1 and 2 contained a fair supply of phosphate; the seed bed was

Table 6. *Swede experiments*

	No. fertilizer	Fertilizers*						Significant difference ($P=0.05$)	
		A		B		One band below seed	In drills with seed		
		Two bands 1½ in. from seed	Two bands 3½ in. from seed	Two bands 1½ in. from seed	One band below seed				
(1) Jealott's Hill, 1937	Yield	14.0	13.3	—	13.2	—	—	0.79	
	No. of roots	244	232	—	204	—	240	22	
(2) Jealott's Hill, 1938	Yield	8.9	9.7	—	8.1	—	8.8	0.6	
	Adjusted yield	8.7	9.4	—	8.7	—	8.7	0.6	
	No. of roots	231	233	—	180	—	215	20	
(3) Dorchester, 1938	Yield	8.6	11.4	—	11.3	—	10.0	1.9	
	Adjusted yield	8.3	11.5	—	11.4	—	9.9	2.1	
	No. of roots	203	237	—	236	—	230	25	
(4) Puddletown, 1938	Yield	12.0	16.4	—	18.4	—	16.8	2.5	
	Adjusted yield	11.8	16.3	—	18.5	—	16.9	2.5	
	No. of roots	220	220	—	234	—	236	16	
(5) Blandford, 1938	Yield	5.9	7.6	—	7.9	—	7.4	0.9	
	Adjusted yield	6.4	7.3	—	7.7	—	7.4	0.7	
	No. of roots	182	209	—	207	—	201	22	
(6) Jealott's Hill, 1939	Yield	6.5	8.4	10.5	8.4	6.3	8.2	1.8	
	Adjusted yield	5.4	9.3	9.0	8.4	9.0	7.3	1.6	
	No. of roots	187	137	196	159	91	184	22	
(7) Bere Regis, 1939	Yield	7.7	11.7	10.0	13.0	—	10.1	1.7	
	Adjusted yield	8.4	11.4	9.9	12.4	—	10.4	1.6	
	No. of roots	179	209	205	221	—	191	24	

Yields: tons per acre. Number of roots: hundreds per acre at lifting time.

* ? cwt. per acre of a granular fertilizer containing 7.0% N, 24.0% water-soluble P_2O_5 , 4.4% insoluble P_2O_5 , and 10.0% K_2O .

good at all four centres. In the one case where fertilizer was sown in the drills, with the seed, the stand of plant was very poor and the yield was low.

The results of three experiments on mangolds and three on sugar beet are given in Tables 7 and 8. In three out of six experiments, broadcasting the fertilizer gave lower yields than one or other of the methods of restricted placement. In two cases out of four, fertilizer in bands $3\frac{1}{2}$ in. from the seed gave lower yields than when in bands $1\frac{1}{2}$ in. from the seed. When sown below the seed, fertilizer gave lower yields than when sown at the sides of the seed in three out of six trials. In two cases out of four, sowing fertilizer in the drills with the seed gave very poor results.

Owing to the trouble experienced in 1937-9 with clods jamming between the fertilizer coulters, even when they were staggered, sowing fertilizer in one band $1\frac{1}{2}$ in. away from the seed, was tried in 1940. This gave the highest yield in both experiments.

DISCUSSION

The results presented confirm American experience that higher crop yields are obtained by applying fertilizers in restricted zones or bands than by broadcasting and that no one method of restricted application is universally the best.

Sowing fertilizers in the drills with the seed of swedes, mangolds and sugar beet, often impairs braiding and gives poor yields, but good results are obtained on soils very deficient in one or more nutrients since the beneficial effect of the nutrient far outweighs its potential harmful effect. This method cannot be recommended for general use, since the risk involved is serious and other methods of application give at least as good results without any risk. It can, however, be recommended where superphosphate is used alone on soils very deficient in phosphate.

On most soils, sowing fertilizer in a band below, but not in contact with the seed, is likely to lead to a poor stand of plant and low yields, but on soils very deficient in one or more nutrients (usually phosphate), it seems to lead to the highest yields.

Sowing fertilizer in bands at both sides of the seed, or in a single band at one side, is usually most satisfactory. It has the advantage of concentrating fertilizer near the seed, without risk of impaired braiding. The lateral distance of the bands should not be as much as $3\frac{1}{2}$ in.; $1\frac{1}{2}$ in. proved better. When sowing fertilizer at both sides of the seed in bands $1\frac{1}{2}$ in. away, difficulty was experienced with the experimental drill on

Table 7. *Mangold experiments*

		Fertilizer*						Significant difference (P = 0.05)
		A		B		In drills		
		No fertilizer	Two bands 1½ in. from seed	Two bands 3½ in. from seed	One band below seed	One band with seed	One band 1½ in. from seed	Broadcast
(1) Jealott's Hill, 1938	Yield	8.1	10.6	—	9.6	7.0	—	9.8
	Adjusted yield	7.7	9.8	—	9.7	7.9	—	9.9
	No. of roots	158	164	—	147	131	—	146
(2) Jealott's Hill, 1939	Yield	9.8	16.1	14.0	16.5	15.8	—	14.4
	Adjusted yield	10.3	16.0	14.8	16.0	15.2	—	14.2
	No. of roots	198	215	187	201	233	—	247
(3) Jealott's Hill, 1940	Yield	17.5	24.7	24.7	20.4	—	27.1	21.8
	No. of roots	158	192	192	144	—	221	184
	Yield: tons per acre of roots.	Number of roots: hundreds per acre at lifting time.						25

* 3 cwt. per acre of a granular fertilizer containing 12.4% N, 12.4% water-soluble P_2O_5 and 14.9% K_2O .

Table 8. *Sugar-beet experiments*

		Fertilizer*						Significant difference (P = 0.05)
		A		B		In drills		
		No fertilizer	Two bands 1½ in. from seed	Two bands 3½ in. from seed	One band below seed	One band with seed	One band 1½ in. from seed	Broadcast
(1) 1938	Yield	5.4	5.9	—	4.6	4.0	—	5.9
	Adjusted yield	5.1	5.5	—	5.3	4.5	—	5.5
	No. of roots	199	203	—	169	166	—	203
(2) 1939	Yield	7.0	11.0	9.4	11.9	11.7	—	11.3
	Adjusted yield	7.2	11.5	10.7	11.9	10.7	—	10.5
	No. of roots	219	206	180	224	262	—	255
(3) 1940	Yield	7.8	10.6	9.7	8.8	—	11.3	9.2
	No. of roots	206	221	218	168	—	223	201
	Yield: tons of washed beet per acre.	Number of roots: hundreds per acre at lifting time.						24

* 3 cwt. per acre of a granular fertilizer containing 12.4% N, 12.4% water-soluble P_2O_5 and 14.9% K_2O .

cloddy seed beds owing to the clods jamming between the coulters. Staggering the coulters greatly reduces the trouble but does not eliminate it on very cloddy seed beds. A far simpler solution to the problem is to sow the fertilizer in a single band at one side of the seed, and this gave very good results. The types of drill now in use which sow fertilizer below the seed could easily be altered to sow it at one side of the seed.

The fact that fertilizer in a band directly below the seed, but separated from it by an inch of soil sometimes gives a poor stand of plant, whereas fertilizer in bands at the same depth but distant laterally one inch from the seed does not, calls for an explanation. The primary rootlet grows downwards and soon comes in contact with fertilizer sown an inch below, whereas the first contact with fertilizer sown at the sides of the seed is probably by secondary roots. If the primary root is damaged the seedling may die but any harm to the secondary roots is less important as fresh ones can be formed.

Two aspects of the problem of applying fertilizers in bands, need further study. The first is the depth of the fertilizer below the seed and the lateral distance of the bands from the seed. The second is the effect of zonal placement of fertilizers for one crop, on the succeeding crop. It might be imagined that zonal placement of fertilizers for one crop would be reflected in zonal growth of the succeeding crop, but the root range of most crops is extensive and this, coupled with the mixing caused by cultivations, tends to eliminate such an effect. No zoning of the succeeding crop was noticed on any of the experimental areas and none is apparent in those districts where it is usual to sow superphosphate in the drills with swede and kale seed. However unfortunate it may be from some points of view, the gradual disappearance of fixed rotations and the consequent tendency to fertilize each crop on its own merits also react against any residual zonal effect.

Ideally, the placement of N, P and K should be considered separately. While this would simplify interpretation of experimental results, it is perhaps of more academic than practical interest. Some very great advantage would have to accrue from sowing N, P and K in different ways before farmers could adopt such a practice as the necessary machinery would be complicated and costly.

SUMMARY AND CONCLUSIONS

Evidence is adduced to show that sowing the fertilizer in restricted zones or bands gives higher yields of swedes, sugar beet and mangolds than broadcasting and harrowing in fertilizers.

Sowing fertilizer in bands at one or both sides of the seed is the most satisfactory method of application. The bands should be about $1\frac{1}{2}$ in. from the seed and an inch or so below it. Placing the bands much further away than $1\frac{1}{2}$ in. gives inferior results.

Sowing fertilizer in bands at both sides of the seed involves risk of bad sowing, as, on bad seed beds, clods may jam between the fertilizer coulters even if they are staggered. Sowing fertilizer in a single band at the side of the seed obviates this difficulty and sometimes gives higher yields. It is recommended that for swedes, mangolds and sugar beet, complete fertilizers should be sown in one band about 1 in. below and distant laterally about $1\frac{1}{2}$ in. from the seed.

On soils very deficient in one or more nutrients, sowing fertilizer in a band below, but not in contact with the seed, gives very good results, but on most soils it is likely to lead to a poor stand of plant and low yields.

Sowing fertilizers in the drills in contact with the seed gives good results on soils acutely deficient in one or more nutrients, but it can only be recommended for specific fertilizers in restricted areas (e.g. superphosphate can be used in this way for swedes on some soils in Wiltshire and Dorset) since it involves serious risk of impaired germination.

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APPENDIX

Through the kind co-operation of Messrs Cashmore and Dawson of the Institute for Research in Agricultural Engineering of Oxford, a drill manufactured by Messrs Knapp of Clanfield, Oxon, was adapted to sow fertilizer in various ways. This is a three-row drill having two hoppers, a forward one for fertilizers and a rear one for seed. As marketed, the drill sows seed and fertilizer down separate coulters, the fertilizer coulters being in front. The relative depths of the two sets of coulters can be varied. The fertilizer part of the drill is based on the roller and moving slide principle and, as with all drills of this type, the roller and slide were not true enough for experimental work. At the suggestion of Messrs Cashmore and Dawson, the space between the slide and roller was blocked up except for one opening 6 in. wide over each set of fertilizer coulters. These openings were controlled by specially attached slides, and the rate of application of fertilizer was made the same for each set of coulters by adjusting these slides. Blocking up most of the space between the roller and main slide resulted in increased accuracy of sowing as the small slides have to be opened much wider than the original main slide for a given rate of application. To sow fertilizer at both sides of the seed, pairs of coulters were fixed in front of the seed coulters. Equal amounts of fertilizer were led into individual coulters by having the centre of each small adjustable slide immediately above the point of a wedge. When sowing fertilizer from single coulters either below or at one side of the seed, the pairs of fertilizer delivery tubes were led into the single coulters. To sow fertilizer in the drills with the seed, the fertilizer delivery tubes and the seed tube were led into single coulters. The fertilizer bands were in all cases about half to one inch wide. When sown in one band below the seed or in one or two bands at the side of the seed, the fertilizer was placed about one inch below the level of the seed.

SOIL PROPERTIES IN RELATION TO THE OCCURRENCE OF GRASS SICKNESS IN HORSES

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INTENSIVE research work into the cause of grass sickness in horses has been carried out during recent years, and workers in practically every sphere of biological science have been asked to contribute their knowledge to the elucidation of the aetiology of this condition. Although in the past both the soil and the herbage have been examined in order to find out whether any obvious abnormality existed in grass sickness areas, no organized approach to the problem had been attempted from the soil side. In 1938 the Institute was asked by the Animal Diseases Research Association to collaborate in a survey of grass sickness in Aberdeenshire and to make a comprehensive examination of the soil of a number of farms on which grass sickness occurred that year. The aim of the soil examination was to discover whether there existed a relationship between soil properties and the incidence of grass sickness.

A selection was made of sixty-three farms in Aberdeenshire on which cases of grass sickness occurred during the summer of 1938, and in the majority of cases the soil samples were obtained from the fields within a few days of the horses' contracting the disease. Most of the fields were under temporary pasture but there were also instances of older or permanent pasture involved. The farms, in districts of varying altitude, with varying geological formations and varying fertility, may be regarded as representative of much of the North of Scotland. Samples of soil representative of the top 2 in., in which are concentrated the bulk of the pasture roots, the surface soil to the ordinary cultivation depth and the subsoil were taken from the various fields in June 1938. It was hoped that a comprehensive examination of these samples would show whether the occurrence of the disease was to be associated with a particular soil condition.

The examination of the samples includes the determination of both readily soluble and reserve plant food materials, exchangeable bases and

hydrogen, mechanical composition, acidity conditions, carbon-nitrogen relationships and in addition the contents of some of the minor elements in the soil. The possibility of the presence of toxic substances, such as sulphides, in the fresh samples was also borne in mind. Unfortunately it was impossible, on account of the lack of facilities at the Institute, to undertake biological and microbiological investigations, although the possible importance of these was recognized. For convenience the various samples from each field will be referred to throughout by the letters C, A and B as follows:

C samples: Representative of the top 2 in. of soil.

A ,, : Representative of the surface soil to the ordinary cultivation depth.

B ,, : Representative of the subsoil, i.e. of that portion of the soil between the depths of approx. 9 and 18 in. below the surface.

Parent materials. Practically all the soils have been formed from glacial drift, which in the majority of cases, however, is similar in mineralogical composition to the rocks which it overlies. Geologically the parent materials in order of relative abundance fall into the following groups: granite, 17; knotted schists and slates, 16; gneiss, 10; diorite, norite and gabbro, 11; fresh-water alluvium, 4; syenite, 2; old red sandstone conglomerate, 2; and serpentine, 1. Although, as might be expected in Aberdeenshire, most of the soils have been derived from acid rocks, there are sufficient basic types and sufficient variation in the geological formations represented to show that the occurrence of grass sickness is not confined to soils of any particular geological origin.

Texture. Texturally there is likewise a considerable variation in the soils examined, as may be seen from the following table in which is summarized the range in mechanical composition.

A mechanical composition of the order of that represented by group II may be regarded as typical of a large proportion of the ordinary agricultural soils in the north-east of Scotland. It will be seen that the greater proportion of the soils examined have contents of the various mineral fractions and of organic matter which fall into this group, which may be regarded as a medium loam, but the disease also occurs to an appreciable extent on the more extreme textural types, including seven humose or peaty soils. There does not appear, therefore, to be any question of a relationship between the incidence of grass sickness and soil texture.

Table 1. *Distribution of soils in groups from results of mechanical analyses.*
The figures in the 'group' columns are percentages of the samples examined

Viz. 78 surface 'A' samples and 19 subsoil 'B' samples.

	Absolute limits of variation	Group I	Group II	Group III
% Coarse sand:	<30	30-50	>50	
A, surface	13-60	25.6	66.7	7.7
B, subsoil	16-75	21.1	47.3	31.6
% Fine sand:	<20	20-30	>30	
A, surface	11-39	10.3	78.2	11.5
B, subsoil	11-43	15.8	52.6	31.6
% Silt:	<10	10-20	>20	
A, surface	5-29	9.0	82.0	9.0
B, subsoil	3-28	26.3	57.9	15.8
% Clay:	<10	10-20	>20	
A, surface	7-26	3.9	88.4	7.7
B, subsoil	6-23	31.6	63.2	5.2
% Loss on ignition:	<8	8-15	>15	
A, surface	6-39	2.6	88.4	9.0
B, subsoil	3-23	57.9	36.9	5.2

Soil acidity, base status and plant food contents. In the following tables details are given of variations in acidity, base contents and other properties of the samples examined. In each case group II in the table may be taken as representative of an average Aberdeenshire soil, which nevertheless is not necessarily entirely satisfactory from the agricultural point of view. In group I the soils may be regarded as 'below average' and in these suitable treatment could be expected to bring about a considerable improvement in their agricultural value. In group III the soils

Table 2. *Soil acidity, base status and plant food contents*

	Absolute limits of variation	Group I	Group II	Group III
Range of pH values	<5.5	5.5-8.2	>6.2
C	5.0-6.9	15.3	76.4	8.3
A	4.9-6.05	28.8	71.2	0
B	5.2-6.65	12.3	80.0	7.7
Adsorption capacity as m.e./100 g. oven-dry soil	<14	14-22	>22
C	7.7-52.2	19.4	68.1	12.5
A	7.8-52.6	21.3	62.5	16.2
B	2.3-36.0	60.0	37.0	3.0
Percentage saturation of soil with bases	...	<30	30-60	>60
C	22-81	13.9	80.5	5.6
A	21-72	27.5	67.5	5.0
B	10-88	29.2	53.8	17.0

Table 2 (continued)

	Absolute limits of variation	Group I	Group II	Group III
Exchangeable hydrogen m.e./100 g. oven-dry soil	>12	12-8	<8
C	2.7-39.6	26.4	51.4	22.2
A	3.2-38.2	30.0	47.5	22.5
B	1.5-21.6	12.3	30.8	56.9
Exchangeable Ca m.e./100 g. oven-dry soil	<3.6	3.6-7.2	>7.2
C	2.4-16.8	9.7	69.5	20.8
A	2.5-12.1	13.8	70.0	16.2
B	1.1-12.9	49.2	40.0	10.8
Exchangeable Mg m.e./100 g. oven-dry soil	<0.4	0.4-1.0	>1.0
C	0.3-3.3	6.9	57.0	36.1
A	0.2-2.9	18.8	65.0	16.2
B	0.1-9.5	29.2	47.7	23.1
Exchangeable Ca/Mg ratios (equivalents)	<6	6-12	>12
C	1.8-18	32.0	62.5	5.5
A	2.0-39	23.8	58.7	17.5
B	0.7-25	36.9	46.2	16.9
Exchangeable Ca/K ratios (equivalents)	<20	20-40	>40
C	7-110	26.4	57	16.6
A	9-100	13.7	60	26.3
B	4-190	12.3	46.2	41.5
Exchangeable Ca/Sr ratios (equivalents)	<400	400-800	>800
C	180-1400	18.1	72.2	9.7
A	220-1100	15.0	71.3	13.7
B	270-1400	18.5	72.3	9.2
Exchangeable Ca/Mn ratios (equivalents)	<125	125-250	>250
C	38-1200	39	37.5	23.5
A	38-820	32.5	46.3	21.2
B	80-2700	1.5	16.9	81.6
Exchangeable Mg/Mn ratios (equivalents)	<15	15-30	>30
C	7-160	26.4	38.9	34.7
A	3-120	27.5	47.5	25
B	9-1200	6.1	18.5	75.4
Readily soluble phosphate mg. P_2O_5 /100 g. oven-dry soil	<5	5-12	>12
C	2-60	9.7	54.2	36.1
A	2-34	23.7	48.8	27.5
B	Trace-35	30.8	40.0	29.2
Readily soluble potash mg. K_2O /100 g. oven-dry soil	<5	5-10	>10
C	4-35	1.4	52.8	45.8
A	4-30	7.5	72.5	20.0
B	1-13	58.5	38.4	3.1

are 'above average' and likely to be satisfactory for agricultural purposes. It will be remembered, of course, that a soil may be low in one constituent and high in another, and a soil which for instance comes into the 'satisfactory' group on one count may be classed in another group on the basis of its content of another constituent. The extreme limits of values

obtained are noted separately, and in the tables the figures in the 'group' columns are expressed as percentages of the number of samples examined, viz. 72 'C' samples, 80 'A' samples and 65 'B' samples, unless otherwise stated.

Table 3. *Difficulty soluble or 'reserve' plant food materials (conc. HCl extract) for 72 'C', 80 'A' and 20 'B' samples*

	Absolute limits of variation	Group I	Group II	Group III
Phosphate, mg. P_2O_5 /100 g. oven-dry soil	... <125	125-250	>250	
C	43-590	20.8	62.5	16.7
A	60-640	28.7	53.8	17.5
B	20-280	63.6	31.8	4.6
Potash, mg. K_2O /100 g. oven-dry soil	... <300	300-600	>600	
C	160-820	13.8	68.1	18.1
A	130-870	15.0	66.2	18.8
B	260-1460	20.0	55.0	25.0
Lime, mg. CaO /100 g. oven-dry soil	... <300	300-600	>600	
C	210-1270	13.8	62.5	23.7
A	200-1090	17.5	57.5	25.0
B	180-1100	43.5	30.4	26.1
Magnesia, mg. MgO /100 g. oven-dry soil	... <400	400-800	>800	
C	76-1210	19.4	63.9	16.7
A	150-2200	22.5	60.0	17.5
B	170-2150	20.0	55.0	25.0
Manganese oxide, mg. MnO /100 g. oven-dry soil	... <40	40-80	>80	
C	16-500	18.1	55.5	26.4
A	15-230	22.5	51.3	26.2
B	21-150	50.0	30.0	20.0

Table 4. *Carbon : nitrogen relationships
(for 33 'C' samples and 36 'A' samples)*

	Absolute limits of variation	Group I	Group II	Group III
Carbon, C as % oven-dry soil	... <3.0	3.0-6.4	>6.4	
C	2.7-18.3	15.2	69.6	15.2
A	2.4-22.6	8.3	75.0	16.7
Nitrogen, N as % oven-dry soil	... <0.25	0.25-0.4	>0.4	
C	0.17-0.75	15.2	63.6	21.2
A	0.20-1.15	11.1	77.8	11.1
C/N ratios	... <12	12-16	>16	
C	10.9-24.4	18.2	63.6	18.2
A	11.8-27.0	2.8	75.0	22.2

pH values (Table 2). For an ordinary six-course rotation of cereals, roots, cereals, hay and two years' pasture, such as is followed on the great majority of the farms sampled, a soil *pH* value of about 6.2 or over is highly satisfactory. Soils with values between 5.5 and 6.2 are becoming

slightly too acid for the best results to be obtained, particularly with hay and pasture crops, and on these soils the application of some lime is to be considered advisable, if the soil is to be kept from becoming markedly acid. Soils with pH values less than 5.5 are to be regarded as becoming too acid for the best results to be obtained with hay or pasture crops. From the table it will be seen that the majority of the soils fall into group II, i.e. the group which is becoming slightly too acid. In none of the ordinary surface 'A' samples can the acidity conditions be considered highly satisfactory and almost one-third of the samples fall into the markedly acid group. There is a general tendency for the top 2 in. or 'C' samples to be less acid than the 'A' samples, a result which could be expected at the height of the growing season, when the solvent action of root excretions is likely to be at its greatest. As is to be expected in a region where the soils belong mostly to the podsolic type the subsoils tend to be less acid than the corresponding surface soils.

Base status (Table 2). From the table it will be seen that the adsorption capacities of the 'C' and 'A' samples are similar. In the subsoil, where the material is naturally less weathered, the adsorption capacities are smaller and 60% of the samples have capacities of less than 14 m.e. per 100 g. The majority of the surface soils again fall into group II and the remainder are about equally divided between the relatively high group III and the low group I.

Although there is no simple relationship between pH value and percentage saturation, there is broad general agreement between them. The 'C' samples tend to have a slightly higher percentage saturation than the 'A' samples, and in only a very small proportion of the surface soils (cf. Group III) could an increase in base content be considered unnecessary. From the figures for exchangeable hydrogen it will be seen that about half the soils fall into group II, i.e. have a hydrogen content which is slightly too high, and there are more surface soils in the unsatisfactory group I than in the more satisfactory group III. The differences between the hydrogen contents of the 'C' and 'A' samples are less pronounced than the corresponding differences in pH values and percentage saturation with bases. In both percentage saturation and hydrogen content the 'B' samples are more irregular than the corresponding surface soils. More than half of the 'B' samples fall into group III with low contents of exchangeable hydrogen. The lack of a correspondingly high proportion with high percentage saturation with bases is explained, when it is borne in mind that the subsoil samples have much lower base exchange capacities than the surface soils.

Exchangeable or readily soluble lime, magnesia and potash (Table 2). Of the basic cations which occupy the exchange or adsorbing complex in a soil, calcium is generally the most abundant. From the results for exchangeable Ca it is seen that the majority of the surface soils fall into the average group II and in these the lime content could, from the agricultural point of view, be increased slightly with advantage. The 'C' samples are slightly richer in Ca than 'A'. It will be noted that a high proportion of the 'B' or subsoil samples are low in Ca. There are likewise high magnesia contents in only a few of the subsoils, and the lower acidity of the 'B' samples referred to above appears therefore to be due not to a large content of Ca or Mg, but to their relatively low hydrogen contents and their low adsorption capacities. Figures are given for exchangeable magnesia, as next to calcium, magnesium is generally the most abundant of the divalent cations in soils. It is of interest to note that the 'C' samples are richer in Mg than the 'A' samples and that the relative differences in the Mg contents of the 'C' and 'A' samples are more pronounced than those for Ca.

Before considering the cation ratios in the soil it is convenient also to consider potassium. In Table 2 the soils are grouped according to their contents of readily soluble potash. This is determined in a 0.5 N CH_3COOH extract by the method used in soil advisory work at the Institute. Separate estimations were made of exchangeable and readily soluble cations and the results generally showed good agreement. Soils in group III may be regarded as being fairly well supplied with potash; those in group I are relatively low in potash and for those in group II the potash supplies could with advantage be increased slightly. The majority of the soils are thus slightly low in potash and a relatively small proportion are very low. The 'C' samples are markedly richer in potash than the 'A' samples, most probably as a result of the action of plant roots already referred to. The subsoils are markedly lower in potash than the corresponding surface soils.

Exchangeable cation ratios (Table 2). For satisfactory plant growth it is generally recognized that certain minimum amounts of various elements must be present, but apart from this most plants are able to adapt themselves to a wide range of concentrations of various salts. Although there is general agreement amongst investigators on the necessity for a suitable nutrient balance, if optimum growth is to be obtained, there do not appear to be any fixed optimum ratios, and the possible effects of antagonism between ions are by no means fully understood. In the characterization of the soils in the present investigation it was decided to

determine the exchangeable cation ratios for some of the elements commonly found in soils, with a view to finding whether there were any strikingly abnormal ratios with which the incidence of grass sickness might possibly be related.

Whilst there are available many results on acidity conditions, and ordinary nutrient relationships for different Scottish soils, there is comparatively little information on their magnesium, manganese, strontium, carbon and nitrogen contents. With the results for Mg, Mn, Sr and to a somewhat lesser extent C and N, the grouping is, as far as is possible from the limited general data available, in keeping with the three main classes of 'relatively low'—group I, 'fairly average'—group II and 'relatively high' group III.

Ca/Mg. A high ratio may be due either to a high Ca or a low Mg and conversely a low ratio may mean either low Ca or high Mg. (Similar considerations will, of course, apply to the other cation ratios.) The 'B' and 'C' samples both tend to have lower Ca/Mg ratios than the 'A' samples. The lower ratios for 'C' as compared with 'A' are due mainly to the relatively higher Mg contents of the 'C' samples. Of the surface 'C' and 'A' samples the majority fall into the average group, whilst in the subsoils the values are more widely distributed.

Ca/K. Here the values are lower for the 'C' than for the 'A' samples, and there is a large proportion of the subsoil samples with high ratios. The lower values in the 'C' samples are due chiefly to their higher potassium contents, whereas the high ratios in the subsoil are due mainly to low potash. The majority of the surface samples again fall into the average group.

Ca/Sr. The most noticeable feature is the similarity in distribution of the three sets of samples in the three groups. Variations in cation ratios will occur where the cations have differing properties and consequently behave differently in the soil. The results confirm observations by Mitchell (1937), and indicate that in spite of the much greater absolute amount of calcium in the soil, calcium and strontium, unlike Ca and Mg for instance, behave in like manner and are transported in the soil either together or in roughly equivalent amounts. The majority of the samples again fall into the intermediate group.

Ca/Mn. Here the 'C' samples tend to have slightly lower ratios than the 'A' samples, whereas the majority of the 'B' samples have high ratios. The 'C' samples are richer in calcium and also slightly richer in manganese than the 'A'. In the 'B' samples the manganese contents are low and the ratios consequently high. In the surface samples the range of

distribution of the Ca/Mn ratios is wider than for the other cation ratios and the number of soils in all three groups is fairly high.

Mg/Mn. The magnesium : manganese ratios are somewhat higher in 'C' than in 'A' as a result chiefly of the relatively higher Mg contents of the 'C' samples, and the range of distribution as with Ca/Mn is again a wide one. There is likewise the similarity to Ca/Mn distribution in the large percentage of subsoils with a high Mg/Mn ratio.

Readily soluble phosphate (Table 2). The values for phosphate are obtained in the 0.5 N CH_3COOH extract already referred to in connection with the estimation of readily soluble potash, and the grouping is as for potash. The phosphate and potash supplies are of a like order in the various samples, but with phosphate the values are more divergent and the differences rather less pronounced. The top 2 in. are generally slightly richer than the general surface soil and in the majority of the soils the phosphate supplies could be increased with advantage.

Difficulty soluble or reserve plant foods. Details are given in Table 3 of the probable reserves of phosphate, potash, lime, magnesia and manganese in the surface soil samples and in selected subsoil samples, as determined by means of extraction with concentrated HCl.

Phosphate. There is comparatively little difference in the 'C' and 'A' samples and the majority fall into the average group, with a slightly greater percentage in the low than in the high group. By far the biggest percentage of the subsoil samples falls into group I, but it is to be noted here that the grouping is based on what is average for an ordinary surface soil. With phosphate which is not readily washed out of the soil and of which there is not a big natural reserve in most Scottish soils, the above subsoil contents of P_2O_5 in relation to the contents of the corresponding surface soils may be regarded as quite normal.

Potash. Most of the surface soils fall into the average group and there is comparatively little difference in the potash contents of the 'C' and 'A' samples. As compared with phosphate supplies, the potash contents of the subsoils are relatively much higher. This agrees with the general observation that the natural reserves of potash in the form of minerals is much greater than that of phosphate in most Scottish soils.

Lime. The 'C' and 'A' samples contain approximately equal reserves of lime and the greatest proportion of the samples are again in the average group. The subsoils are relatively lower in lime reserves than the surface soils.

Magnesia. The distribution of the magnesia contents is similar to that for potash and much less variable than for phosphate or lime.

Manganese. In manganese contents the 'C' and 'A' samples are again similar and about half of them fall into the average group. The subsoils generally are lower in manganese than the corresponding surface soils.

As is to be expected the variations in the contents particularly of the 'C' and 'A' samples in difficultly soluble or reserve food materials are much less pronounced than the corresponding variations in readily soluble or exchangeable amounts. The results for 'reserve' plant foods as outlined above show no abnormality likely to account for the occurrence of grass sickness.

Carbon : nitrogen relationships (Table 4). Several investigators attach considerable importance to the study of carbon : nitrogen ratios in the characterization of soils. A selection was therefore made of 36 'A' and 33 'C' samples for carbon and nitrogen determinations in order to see whether abnormal carbon : nitrogen ratios obtained. The significance of C/N ratio in soil in relation to plant growth is by no means fully understood and it is doubtful whether it is a factor of much importance. For Rothamsted soils Russell (1937) gives a C/N ratio in the neighbourhood of 10 as being common in ordinary fertile soils, and reports the amount of carbon as being of the order of 1-3 % with corresponding nitrogen figures of 0.1-0.25 %. In these Aberdeenshire soils the mean figures for both carbon and nitrogen, and particularly carbon, are considerably higher, and about three quarters of the surface samples examined have C/N ratios of 12 to 16. This is to be expected in a region where, as in Aberdeenshire, many of the soils originally had a thin covering of peat and have been under cultivation for a relatively shorter period than the Rothamsted ones. Another contributory factor is possibly a lower mean annual temperature with consequent slower decomposition of the organic matter in the soil. It will be noted that there is a relatively wide range in the contents of both carbon and nitrogen. It was pointed out that some of the ordinary surface soils were classed as humose or peaty, and it is these which are responsible for a few carbon and nitrogen values which are well above the average. The 'C' samples tend to have slightly lower C/N ratios than the 'A' samples, presumably as a result of more rapid decomposition in the upper than in the lower layers of the soil.

In addition to the foregoing, the 'C' samples were examined qualitatively for sulphides and for chlorides. The tests for sulphides proved negative and in none of the samples was there evidence of an abnormal chloride concentration.

SUMMARY

Any conclusions drawn from the foregoing results will of course apply only to the necessarily limited range of soils in one county. This range is limited geologically and the Aberdeenshire soils belong largely to the class of light to medium acid soils of low base status. The results for these soils indicate that:

(a) The occurrence of grass sickness is not confined to soils of any particular geological origin.

(b) The range of textural conditions in the soils examined is a wide one covering loams, sands and peaty types with a few moderately heavy soils. There does not appear to be any relationship between soil texture and the incidence of grass sickness.

(c) The soils examined all fall into the class of acid soils and in only a very few—about 5 to 10 %—could the addition of lime be considered unnecessary from the general agricultural point of view. As against this there are no striking abnormalities in the ratios of exchangeable or readily soluble cations in the soils, which might serve as a clue to the occurrence of grass sickness. Although little is known of the actual magnesium, manganese and strontium requirements of hay and pasture plants, the range of values covered in the soils examined is sufficiently wide to make it very unlikely that there is any relationship between the incidence of grass sickness and the magnesium, manganese or strontium contents of the soil. In view of the relatively widespread acidity in the soils examined, the possibility of a relationship between the occurrence of grass sickness and soil acidity has to be borne in mind. It would be of value to find out if grass sickness occurs to an appreciable extent on limestone soils, or on soils which have been systematically limed and which have *pH* values in the neighbourhood of 7.

(d) The majority of the soils are, from the general agricultural point of view, somewhat low in readily soluble or available potash and phosphate, but against this about 30 % of the soils have satisfactory phosphate contents and about 20 % of the ordinary surface soils and 46 % of the top 2 in. samples, in which occur the bulk of the plant roots, have satisfactory potash contents. It does not appear likely therefore that the occurrence of grass sickness is to be related directly to the phosphate and potash contents of the soil. As far as reserves of the common plant foods are concerned there is likewise no abnormality which could account for the disease.

(e) The average values for carbon and nitrogen and for carbon :

nitrogen ratios in the soils examined are higher than those recorded for cultivated soils in S.E. England. In view of the differences in climatic conditions and in the origin of the soils this, however, is not unexpected and the range of values covered is sufficiently wide to make the possibility of correlation between incidence of grass sickness and carbon : nitrogen relationships in a soil unlikely.

Further results on pasture samples and on the contents of certain of the minor elements in selected soil samples will be reported later.

The writer acknowledges with thanks the assistance of Miss A. J. Preddy, who carried out most of the chemical determinations, and of Dr R. L. Mitchell for spectrographic determinations by the Lundegardh method of most of the alkali and alkaline earth contents.

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OBSERVATIONS ON THE HAEMOGLOBIN LEVEL OF COWS AND SHEEP

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THE most extensive data published on the level of haemoglobin in the blood of cattle and sheep have been obtained in America and the British Dominions. Anderson *et al.* (1930) found haemoglobin values of female cattle to be higher than those of male. McCay (1931) reported the opposite, mean values being 10.9 g. per 100 ml. of blood for cows and 12.8 g. for bulls. His data on cows showed no correlation between haemoglobin level and breed, milk yield, fat content of the milk or stage of lactation, and that the average haemoglobin value of a group of seventy-four cows was not materially different when the cows were stall-fed and after two months at pasture. Brooks & Hughes (1932), in a study of 355 samples from 297 head of dairy cattle, found a mean of 10.96 g. per 100 ml. and stated that age, breed and prolonged fasting did not influence blood haemoglobin, that the individual did not vary much from day to day, and that bulls gave the highest values. Miller (1932-3) reported a range of 9.9-14.2 g., average 12.0 g., for adult cattle, and a range of 9.6-18.1 g. with average 12.0 g. per 100 ml. in younger cattle, and associated an observed progressive anaemia with Johne's disease. Neal & Becker (1933) reported that clinically healthy cattle in a 'salt-sick' area (trace element deficiency) showed a range of 7.5-14.0 g. per 100 ml. with values ranging from 10.96 ± 1.54 to 11.06 ± 1.40 g. in different individuals bled several times over a year, while 'salt-sick' cattle showed values as low as 3.0 g. in certain cows which subsequently recovered after iron and copper therapy. A value even as low as 1.4 g. was found in a 'salt-sick' calf just before death. In the Philippine Islands, Manresa *et al.* (1940) found average values of 9.43 and 9.87 g. in native breeds and 6.76 and 6.88 g. in Herefords and Holstein-Friesians imported from U.S.A. They considered that the high temperature and humidity of the district tended to lower the haemoglobin of imported pedigree cattle, but other possible factors were perhaps not fully considered.

Filmer (1933) reported an average value of 15.0 g. per 100 ml. for young Merino sheep. In the *Report C.S.I.R. Commonwealth of Australia* (1933) values for three flocks of Merino sheep are quoted as (a) 12.2-15.2 g., average

13.6 g., per 100 ml. of blood, (b) a mean of 10.3 g., and (c) mean values of 11.4 g. for young sheep and 12.2 g. for aged ewes with lambs at foot. Hamersma (1934), in seasonal studies on small groups of Merino sheep in South Africa, found values ranging from 9.3 to 17.5 g. in lambs and 10.1 to 23.2 g. in ewes. Bennetts & Chapman (1937) give the normal values for Merino ewes as 11-12 g. per 100 ml., while Marston & Macdonald (1938) give the figures for similar sheep as 10-11 g. Underwood *et al.* (1939) give the mean value for Merino ewes as 10.8 g.

In view of the variations in the haemoglobin level reported in various parts of the world, it is considered worth while to publish the frequency distribution of random data from a large number of determinations of haemoglobin in the blood of cattle and sheep from all over England and Wales, incidentally acquired in connexion with the diagnostic service and experimental work of this Laboratory. The determinations were made over the years 1936-40 and the same technique was used throughout. The method adopted was the well-known acid haematin estimation using a standardized Newcomer disk. Sampling of blood was from the jugular vein in practically all cases.

It should be noted that, in sheep, helminth infection to a greater or lesser degree is almost universal, and that Taylor (1938) regards the mere presence of worms as of little pathological significance until the numbers rise to a relatively high level. In cases of extreme infestation anaemia becomes a characteristic feature, and indeed Fourie (1931) regards the damage done by *Haemonchus contortus* as entirely due to the sucking of blood by the parasite. Hence in any studies of so-called normal level of haemoglobin in sheep it must be remembered that cases of subclinical worm infestation may often be included in a group of apparently healthy sheep, and that values on the lower side of the average are more open to suspicion of deviation from the true normal than are values on the upper side.

RESULTS

Cattle. In Table 1 are presented the data obtained on cows. The protocols comprise 295 clinically normal cows, and 932 cows suffering or suspected of suffering from some form of metabolic disorder, mainly 'grass tetany', a clinical condition in which pronounced hypomagnesaemia is the main biochemical feature, 'milk fever' or post-parturient hypocalcaemia, and 'post-parturient dyspepsia', a clinical state in which ketonaemia, commonly associated with hypoglycaemia, is a prominent feature.

Table 1. *Distribution, actual and percentage, of haemoglobin values in 1227 samples of cattle blood, expressed in g. per 100 ml.*

Haemoglobin values	Clinically normal cows		Cases from metabolic disorders		Cases of long-standing ketosis		All non-ketosis cases	
	Actual	Percentage	Actual	Percentage	Actual	Percentage	Actual	Percentage
5.5- 6.4	—	—	3	0.3	2	1	0.1	
6.5- 7.4	—	—	13	1.4	10	3	0.4	
7.5- 8.4	4	1.4	45	4.8	29	16	2.2	
8.5- 9.4	17	5.7	114	12.2	62	52	7.2	
9.5-10.4	57	19.3	161	17.3	71	90	12.5	
10.5-11.4	71	24.2	194	20.8	36	158	21.9	
11.5-12.4	73	24.7	167	17.9	—	167	23.1	
12.5-13.4	32	10.8	118	12.7	—	118	16.3	
13.5-14.4	22	7.5	54	5.8	—	54	7.5	
14.5-15.4	12	4.0	47	5.0	—	47	6.5	
15.5-16.4	5	1.7	12	1.3	—	12	1.6	
16.5-17.4	2	0.7	3	0.3	—	3	0.4	
17.5-18.4	—	—	2	0.2	—	2	0.3	
Totals	295	—	933	—	210	723	—	
9.5-13.4	—	79	—	69	—	—	74	
Under 9.5	—	7	—	19	—	—	10	
Over 13.4	—	14	—	12	—	—	16	

In the clinically normal cow the great majority of values, approximately 80%, lie between 9.5 and 13.4 g. per 100 ml., with approximately 50% within the narrower 10.5-12.4 g. range. In the samples from cases of metabolic disorders the range of values is wider but the distribution is much the same. During analysis of the protocols it was noted that cases of 'bovine hypoglycaemic ketosis' which had been affected for a week or more after initial diagnosis of the disorder had been made usually showed lowered haemoglobin values. When these were removed from the metabolic disorders group the 723 remaining samples showed a very similar range and distribution to that of the clinically healthy cows.

From these data the values for haemoglobin in apparently healthy cows may range as far apart as 8.2 and 17.3 g. per 100 ml., but the probable expectation for normal samples is more likely to be from 9.5 to 13.4 g. with greatest frequency around 11.5 g. It is also evident that such disorders as grass tetany and milk fever have no significant effect on haemoglobin level, but that in cases of chronic ketosis the value is usually below normal expectation. The data for the 210 samples of this last category are, therefore, tabulated separately in column 6. It will be noted that no value exceeds 11.4 g. per 100 ml. and that some values lie below 6.4 g. The actual range was 6.2-10.9 g. with 50% of values below 9.5 g. and 20% below 8.5 g.

The metabolic disorder of which the ketosis is a symptom is most

commonly detectable clinically about a fortnight after calving and frequently clears up of its own accord, but chronic cases may not be reported for diagnosis until considerably later and then be found to be in animals which do not usually maintain high condition. It is clear from the case records supplied by those practitioners who forwarded an adequate series of blood samples that clinical recovery and the disappearance of ketonaemia may considerably precede the return of haemoglobin to normal values.

Sheep. Table 2 gives the available data for three groups of apparently normal sheep and two smaller random groupings of individuals diagnosed as suffering from 'pregnancy toxæmia', a disorder occurring several weeks before lambing and characterized, *inter alia*, by pronounced ketonaemia and frequently by hypoglycaemia, and from 'Border Pining', the name given in Northumberland to a wasting disease suspected of being due to a combination of helminth infection and specific malnutrition, the latter probably due to deficiency of cobalt and/or other trace elements.

In the clinically normal sheep of mixed breeds bled at Weybridge the greater part of the values, approximately 83 %, lie between 9.5 and 13.4 g.

Table 2. *Distribution, actual and percentage, of haemoglobin values in 1291 samples of sheep blood, expressed in g. per 100 ml.*

Haemo- globin values	Normal sheep, Weybridge		North- umberland lambs 5-17 months old		North- umberland ewes 3-4 years old		Cases of 'pregnancy toxæmia' in ewes		Cases of 'Border pining'
	Actual	%	Actual	%	Actual	%	Actual	%	
2.5-3.4	—	—	—	—	—	—	—	—	1
3.5-4.4	—	—	—	—	—	—	—	—	1
4.5-5.4	—	—	—	—	—	—	—	—	4
5.5-6.4	—	—	—	—	—	—	—	—	4
6.5-7.4	—	—	2	0.5	4	1.1	—	—	7
7.5-8.4	3	1.1	10	2.7	9	2.5	4	2.6	7
8.5-9.4	11	4.0	32	8.7	38	10.6	16	10.4	6
9.5-10.4	62	22.5	58	15.9	81	22.6	37	24.1	4
10.5-11.4	70	25.4	117	31.9	99	27.7	36	23.6	2
11.5-12.4	73	26.6	83	22.6	72	20.1	27	17.5	—
12.5-13.4	24	8.7	41	11.1	37	10.3	15	9.7	—
13.5-14.4	20	7.3	18	4.8	11	3.1	9	5.8	—
14.5-15.4	9	3.3	7	1.8	5	1.4	3	1.9	—
15.5-16.4	3	1.1	—	—	2	0.6	1	0.6	—
16.5-17.4	—	—	—	—	—	—	5	3.2	—
17.5-18.4	—	—	—	—	—	—	—	—	—
18.5-19.4	—	—	—	—	—	—	1	0.6	—
Totals	275	—	368	—	358	—	154	—	36
9.5-13.4	—	83	—	81	—	81	—	75	—
Under 9.5	—	5	—	12	—	14	—	13	—
Over 13.4	—	12	—	7	—	5	—	12	—

per 100 ml. with somewhat fewer values below than above this range. In the clinically healthy black-faced sheep from Northumberland, both ewes and lambs, again the large majority of values, 81%, lie between 9.5 and 13.4 g., but the greater proportion of the remaining values are lower, possibly due to the inclusion of certain individuals which were subclinical cases of 'pining'. From these data it may be concluded that the normal distribution of values for sheep is very similar to that recorded above for cattle, and that there is no significant difference in level between two age groups, one selected over the range 5-17 months and one aged 3-4 years, when kept under identical conditions.

In the cases diagnosed as 'pregnancy toxæmia' there is no great deviation from the values shown in clinically healthy sheep, the few unusually high values being possibly explained by anhydraemia or circulatory disturbances in the pre-mortæ stages of the disease. In 'Border Pining' a random series of thirty-six cases showed values from 3.2 to 10.9 g. per 100 ml., with 83% below 9.5 g., 66% between 5.5 and 9.4 g., and 17% below 5.5 g.

SUMMARY

The haemoglobin values for the blood of clinically normal cows and ewes over England and Wales are very similar, with a mean value around 11.5 g. per 100 ml. Although the extreme range is wide, about 8-17 g., the distribution of values is such that the common normal range may be taken as 9.5-13.5 g. per 100 ml. There is no striking difference in age groups.

Metabolic disorders such as 'milk fever' and 'grass tetany' in cows and 'pregnancy toxæmia' in ewes have no specific effect on haemoglobin level. Chronic hypoglycaemic ketosis of the dairy cow leads to progressive anaæmia in which levels down to 6 g. per 100 ml. were found. Border or Northumbrian 'pining' of sheep is accompanied by an even more pronounced progressive anaæmia, values as low as 3.2 g. per 100 ml. being encountered.

It is a pleasure to acknowledge the technical assistance of Mr F. J. Salt and the direction and interest of Dr H. H. Green in the main programme from which the protocols were drawn.

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STUDIES IN SOIL CULTIVATION

X. THE RESULTS OF A SIX-YEAR CULTIVATION EXPERIMENT

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THE influence of the tilth of a seed-bed produced by different agricultural implements on the growth and yield of different crops has been studied here by Keen and his co-workers for a number of years. The results are given in the previous nine papers of this series. Up to 1934 only experiments lasting for one or two years had been carried out, but in 1933 a six-year cultivation rotation experiment was started on the Rothamsted Farm which was discontinued after the harvest of 1939. Russell & Keen (1938) and Russell & Mehta (1938) have discussed all the experimental data accumulated on this farm up to 1936, that is, including the results of the first three years of this rotation experiment. The purpose of this paper is to discuss the six years' results.

The general object of the experiment was to find out how far different methods of preparing a seed-bed affected the yield of the crops and the weediness of the land. The three methods chosen consisted in ploughing the land and following by whatever harrowings and rollings were considered necessary (treatment P), grubbing the land with a tractor cultivator followed by harrowings and rollings (treatment G) and obtaining a seed-bed in a single operation by using a small rototiller (treatment R). Each of these primary operations of ploughing, grubbing or rototilling was carried out at two depths of 8 and 4 in., called the deep and shallow treatments, but in order to work to 8 in. with the grubber and rototiller it was necessary to go over the land twice.

The reasons for choosing these three treatments were as follows: The plough cuts a definite furrow and inverts it. This usually, though not necessarily, causes some comminution of the soil surface and so helps to form the necessary seed-bed tilth. But further comminution and consolidation by harrows and rolls is still necessary. The tractor-drawn grubber, or cultivator, breaks up the soil surface without inversion. In

most years, however, it does not leave the land in a suitable condition for a seed-bed and more working with harrows is usually needed. The rototiller, on the other hand, produces a seed-bed in one operation that is usually quite fine enough though it would often be considered too loose. The rototiller achieves this by having tines which, by rotating about a horizontal axis, dig and break up the soil. The experiment was on three adjacent parcels of land which were cropped in the three-course rotation, wheat, mangolds and barley, each parcel carrying a different crop. Each parcel was subdivided into two, by a 40 link (8.05 m.) path down the centre, and each of these were again divided into two. These four divisions of each parcel will be called blocks. Each block was divided into twelve long narrow plots, 139.8 links by 11 links (or 28.12 by 2.21 m.) with a headland on the central path. Twelve different treatments were used, which were the twelve possible combinations of ploughing, rototilling and grubbing, carried out either deep or shallow and dressed with either calcium cyanamide or nitrochalk as the source of nitrogen. The nitrogen was applied to the wheat as a top dressing of 0.3 cwt./acre of N, to the barley a dressing of 0.2 cwt./acre of N was applied at least a week before drilling, and to the mangolds the cyanamide, at the rate of 0.6 cwt./acre of N, was applied at least a week before drilling and half the nitrochalk was applied at this time and the other half at singling. The mangolds also received a basal dressing of 0.75 cwt./acre of P_2O_5 as superphosphate and 1.0 cwt./acre of K_2O as muriate of potash. No other fertilizers were applied.

The four blocks in each parcel were used as follows. On two of the blocks the same cultivation treatment was repeated every year without change, and these blocks form the Continuous Series. On the other two blocks the cultivation treatments rotate. Each plot that is worked deep one year is worked shallow the next, each plot receives nitrochalk for two years and then calcium cyanamide for two years, and on one block the cultivation rotation follows the order plough, rototiller, grubber, and is called rotation A, and on the other the order is plough, grubber, rototiller and is called rotation B. These two cultivation rotations were included so that if, in the following year, there were any residual effects of rototilling or grubbing the land instead of ploughing it, they could be determined.

During the course of the six years the following additions and modifications were made to the cultivation treatments. From 1933 to the harvest year 1936-7 the three primary cultivations of ploughing, rototilling and grubbing were done on the same day, with the exception

of 1936-7 when the wheat ploughed plots were done ten days before the rototilled or grubbed plots. After this date each cultivation was carried out when the farm manager judged it would be most suitable. This resulted in the rototilling being done just before drilling and the ploughing and grubbing being done several days previously.

Trouble was experienced from the very beginning with weeds, and in particular the young plant suffered. To overcome this the wheat stubble was shallow ploughed in the autumn of 1935, and in each subsequent year preparatory to the main spring cultivations for the mangolds. In the last year of the experiment the barley stubble was also shallow ploughed and harrowed preparatory to the main autumn cultivations for the wheat. In the harvest years of 1934-5 and 1936-7 the ploughed and grubbed plots of the barley break were ploughed and grubbed respectively in the winter directly after the mangolds had been carted off, and again in spring just before the seed-bed was prepared.

The general way of preparing the seed-bed after the main cultivations had been done was to harrow and roll the ploughed and grubbed plots once or twice in the direction of cultivation and then to cross-harrow and roll the whole break once across before drilling. The experimental design had, however, a very grave defect in those seasons when it was difficult to obtain a seed-bed, for it did not allow of cross-cultivations being given to only the ploughed and the grubbed plots which needed them and not to the rototilled plots which did not.¹ Only one really difficult season was encountered however. In 1937 the following cultivations had to be given to all plots to obtain a suitable seed-bed for mangolds: spring-tine harrowed across once, lengthways once, and then tractor rolled and drag harrowed once lengthways, across once and then lengthways once, so that the rototilled plots had perforce to have extensive subsequent cultivations to allow a reasonable seed-bed to be prepared on the ploughed and grubbed plots. After this year rototilling was usually deferred until just before drilling, so that the harrows which were used to prepare a suitable seed-bed on the ploughed and grubbed plots could traverse the rototilled plots before they were cultivated.

Various cleaning operations were carried out in the growing crop. The winter wheat was usually harrowed and rolled in March or April and the barley was usually rolled in April or May. In 1934 and 1938 both the barley and wheat plots, and in 1936 the barley plots were hand-

¹ This defect in design was inevitable, as the farm does not possess any close-coupled implements; cross-cultivation with normally hitched implements would have required impossibly wide headlands along the sides of the plots.

hoed in the beginning of May, though only on the wheat plots in 1938 did there seem to be many weeds. If these hoeings had any effect on the crop growth, they would probably tend to reduce the differences due to the various cultivations. The mangold plots were regularly horse-hoed and sometimes hand-hoed as well.

The only other modification that had to be made was in March 1936. The winter wheat had failed, so the whole break was spring-tine harrowed and resown with spring wheat.

The investigation, which was, in part, designed to find out how to conduct long-term cultivation experiments, showed up clearly the importance of the already mentioned fundamental defect, that it was not possible to cross-cultivate selected plots only. This was particularly serious on the grubbed plots, for a much fairer comparison would have been made if the deep grubbed plots had been worked both ways by the grubber and not only lengthways. It also resulted in the rototilled plots having to receive unwanted cross-cultivations which probably were slightly harmful to the tilth.

A further limitation was that neither the grubber nor the rototiller could bury dung, so that none could be added during the experiment. This is not a fundamental limitation of this experiment as there is no evidence that dung is essential for these crops during a six-year experiment on this soil, but the dressings of artificials given were probably too low, bearing in mind the absence of dung, and this is probably the cause of a deterioration of yield that set in, particularly on the barley plots, towards the end of the experiment.

THE EFFECT OF THE TREATMENTS ON THE CROP YIELDS

The yields of each plot have been printed in the *Annual Reports of Rothamsted Experimental Station* for the years 1934-8, and the individual 1939 results will be printed in the next *Report*.

In this section only short summaries of the treatment effects on crop yield will be given, and their form will sometimes be determined by the fact that, since the crop rotation is a three-year one, any appreciable variations due to soil heterogeneity will be eliminated from comparisons between mean yields or mean treatment effects for the first three years and the second three years.

In some tables standard errors have been given to the mean effects of some treatments. If the means only involved plots of the Continuous

Series the variance, i.e. the square of the standard error, of the mean was obtained by dividing the sum of the residual variances of the plot yields over the period of years involved by the suitable factor. This could not be done for treatment means involving plots of the Rotating Series because the residual variances of the plot yields per year cannot be calculated as there was no true replication of these treatments. Approximate standard errors for such means have been calculated for some tables by assuming that, in each year, the variance per plot was the same on the Rotating as on the Continuous Series.

THE LEVEL OF YIELDS

Table 1 gives the mean yields of the three crops for each of the six years, together with the yields of wheat and barley on neighbouring manurial rotation experiments for a comparison.

Table 1. *Level of yields, 1934-9*

Experiment year	Wheat (grain in cwt./acre)		Barley (grain in cwt./acre)		Mangolds (roots in tons/acre) Cultivation
	Cultivation	Two neigh- bouring	Cultivation	Three neigh- bouring	
1934	23.4	27.0	26.2	26.1	35.9
1935	21.2	22.2	35.2	33.9	20.4
1936	21.3	15.7	25.7	27.5	20.7
1937	15.0	16.8	15.0	20.0	19.4
1938	11.8	30.2	16.9	31.0	13.7
1939	25.8	23.2	19.5	33.2	24.6
Mean 1934-6	22.0	21.6	29.0	29.2	25.7
1937-9	17.5	23.4	17.1	28.1	19.2

The yield of wheat on this experiment was similar to that on neighbouring experiments in all years except 1938, when for some unexplained reason there was only a thin plant of wheat which did not tiller well and which looked miserable all through the season. The low yield in 1937 was due to the season and not to the particular conditions on the experimental plots. There has, therefore, been no marked deterioration in the yield of wheat on this experiment compared with neighbouring experiments.

The barley yields, on the other hand, show a marked deterioration of yield in the second three-year period compared with the first, which is not reflected in the yields of barley elsewhere on the farm. This can be seen from Table 1, for whereas the mean barley yields on the neigh-

bouring rotation experiments were 29.2 and 28.1 cwt./acre for the three-year periods 1934-6 and 1937-9 respectively, those on this cultivation experiment were 29.0 and 17.1 cwt./acre, giving a mean drop in yield of about 11 cwt./acre.

There have been no other mangold experiments in the immediate vicinity of this one, though in 1936 and 1937 mean yields of 25 and 21 tons/acre were obtained elsewhere on the farm. The mean yield of 19.2 tons/acre in the second three-year period is lower than 25.7 tons/acre in the first due to the good crop in 1934 and the bad crop in 1938, but owing to this lack of other mangold experiments it is not possible to say definitely how far this reduction is due to a general deterioration of the soil fertility in this experiment or how far it is due to general seasonal factors.

The results of neighbouring sugar-beet experiments suggest that while a part of the lowering is probably due to seasonal effects—for 1934 was also a good and 1938 a bad year—yet a considerable part is due to a lowering of soil fertility presumably due to an inadequate supply of artificials considering that no farmyard manure is supplied. This is brought out in the following table which gives the mean yield of sugar beet in tons/acre in two manorial rotation experiments, one on each side of the cultivation experiment (third column) and in one-year experiments on different fields that were in non-experimental farming for two to three years previously (fourth column):

Crop	Mangolds	Sugar-beet	Sugar-beet
Experiment	Cultivation	Manorial	One year
Mean yield in tons/acre for the period	Rotation	Rotation	
1934-6						25.7	10.6	12.9
1937-9						19.2	7.7	11.7
Percentage reduction in the second three-year period						25	28	10

The yields on the sugar-beet rotational experiments, neither of which had any dung added, follow those of the mangold crop very closely from year to year and show the same apparent deterioration of yield. As the one-year experiments also show an apparent deterioration of yield, part of this is presumably due to seasonal factors. But part may also be due to deterioration, as the mean dressing of sulphate of ammonia in the rotational experiments is only 0.3 cwt. of nitrogen per year, which is much lower than most of the levels used in the majority of the one-year experiments. Thus although no rigorous deductions can be made as to how much, if any, deterioration took place, it is possible that the mangold

yields did show a loss of about 15 % of crop due to an inadequate supply of nitrogen.

Hence the main conclusion to be drawn from these comparisons is that probably the level of manuring on this cultivation experiment was inadequate to maintain for six years the yields of barley and mangolds, though it was adequate for the wheat crop. This result will be discussed in more detail later on in this paper.

**COMPARISON OF NITROCHALK AND CALCIUM CYANAMIDE
AS THE NITROGENOUS FERTILIZER**

A comparison was made between nitrochalk and cyanamide as the form of the nitrogenous fertilizer, since there was a possibility that cyanamide might depress somewhat the growth of weeds. In this event we should expect the plots receiving cyanamide to give a higher mean crop yield or to deteriorate rather slower than those receiving nitrochalk.

The experimental results actually showed that, over the six years, the mean yields on the nitrochalk plots were higher than on the cyanamide plots by the following amounts:

	cwt./acre		cwt./acre		tons/acre
Wheat grain	0.4	Barley grain	0.4	Mangold roots	0.4
Wheat straw	1.6	Barley straw	0.2	Mangold tops	0.3

The only figure calling for any comment is that for the wheat straw, which every year was definitely higher on the nitrochalk plots. This result can be explained in the following way. Watson (1939) found that at Rothamsted a top dressing of sulphate of ammonia applied in March gave a higher straw yield than if applied in April or later, though the yield of grain was hardly affected. The nitrochalk and cyanamide were usually applied in March or early April, and if the cyanamide became available more slowly than the nitrochalk it should be equivalent to a later dressing of available nitrogen and should therefore cause a depression in the straw yield, as found.

There is no evidence that there is any difference of behaviour of nitrochalk and cyanamide in the second three years of the experiment as compared with the first three years. Hence cyanamide has no accumulative beneficial or harmful effect as compared with nitrochalk.

The experiment suggests, therefore, that there is no difference in the response of the crops to cyanamide and nitrochalk unless the time of application of the nitrogen is important.

THE YIELD ON THE PLOUGHED PLOTS

There are four sets of ploughed plots whose yields are worth separating out, the deep and the shallow ploughed plots on the Continuous and on the Rotating Series. The mean yields of these four treatments for the six years are given in Table 2.

Table 2. *Mean crop yields on the ploughed plots*

	Wheat		Barley		Mangolds	
	(cwt./acre)	Grain	(cwt./acre)	Grain	Straw	Roots
Continuous: Deep	22.65	34.94	23.71	29.15	24.54	5.72
	22.82	34.74	24.59	29.47	23.81	5.63
Rotating: Deep	22.28	33.48	23.92	29.02	24.04	5.68
	21.45	32.42	23.32	28.04	22.72	5.45
Continuous minus Rotating	0.87	1.89	0.53	0.78	0.80	0.11
Deep minus Shallow	0.33	0.63	-0.14	0.33	1.03	0.16
Approx. standard error*	0.36	0.68	0.43	0.50	0.44	0.10

* This refers only to the Deep minus Shallow comparison and not to the Continuous minus Rotating, whose standard error is probably considerably larger.

The main results that emerge from this table are that the effect of depth of ploughing is negligible, except possibly for the mangold crop, and that the yield is slightly higher on the Continuous than on the Rotating Series, implying that there may have been a small reduction of yield on those ploughed plots that were rototilled and grubbed in the two previous years. The mean yields of the ploughed plots on rotation A were about the same as on rotation B, so that the harmful residual effect due to using these two types of cultivator, if it existed, did not depend on whether the three-year cultivation rotation was grubber-rototiller-plough or rototiller-grubber-plough. This possible harmful residual effect will be discussed more fully in a later section.

The mean yields of the crops on the ploughed plots of the Continuous Series for the first and second three-year periods of the experiment are given in Table 3.

Table 3. *Mean yields on the Continuously ploughed plots*

	Wheat		Barley		Mangolds	
	(cwt./acre)	Grain	(cwt./acre)	Grain	Straw	Roots
Mean yield: 1934-6	23.83	38.66	30.11	36.13	27.88	5.46
	1936-9	21.63	31.03	18.19	22.48	20.47
Reduction of yield	2.20	7.63	11.92	13.65	7.41	-0.43

This table shows the reduction of yield on the continuously ploughed plots only in contradistinction to Table 1 which is for the whole experiment. The differences between the two tables are small for barley and mangolds, but rather larger for wheat.

Table 4. *The crop response to depth of ploughing in each three-year period*

Series	...	Wheat grain (cwt./acre)		Barley grain (cwt./acre)		Mangold roots (tons/acre)	
		C	R	C	R	C	R
1934-6:							
Deep minus Shallow		-0.77	0.39	0.18	0.50	1.70	0.10
Standard error		0.79		0.99		1.11	
1937-9:							
Deep minus Shallow		0.43	1.29	-1.95	0.71	-0.24	2.54
Standard error		0.66		0.69		0.59	

Series C, continuous; Series R, rotating.

Table 4 shows that this reduction in yield is about the same on the deep as on the shallow ploughed plots of the Continuous Series. But there does seem to have been a response by wheat and mangolds to deep ploughing on the Rotating Series in the second three-year period, probably because the ploughing was following on land that, having been worked mainly by the grubber or rototiller for the preceding two years, was more weedy than the ploughed plots on the Continuous Series. This should not apply to the barley crop, which in fact does not show this response, as it follows the mangold crop which left the land clean, since it was hoed several times. Hence this beneficial effect of deep ploughing is probably due to its having a greater depressing effect on the weed population than the shallow ploughing.

Thus over the six years of the experiment there is no evidence that ploughing below a 4 in. depth confers any benefit to the crop that is reflected in its yield unless the land is dirty, when it may be advantageous to plough deeper. This result is in accord with the general run of experimental results on this farm.

COMPARISON OF THE PLOUGH AND ROTOTILLER

The wheat and mangold yields were definitely lower on the rototilled than on the ploughed plots over the six-year period, while the barley yields were about the same on the deep rototilled plots and a little down on the shallow, as is shown in Table 5. For all the crops, except mangold tops, the yields were higher on the deep rototilled plots than on the

Table 5. *Mean yield of the ploughed and rototilled plots*

	Wheat		Barley		Mangolds	
	(cwt./acre)	Grain	(cwt./acre)	Grain	Straw	(tons/acre)
Mean ploughed, P	22.30	33.90	23.88	28.92	23.78	5.62
Rototilled deep, RD	18.84	30.10	24.26	28.29	22.44	5.19
Rototilled shallow, RS	17.80	28.64	22.54	26.78	21.21	5.26
Rototilled: Continuous Series	18.05	29.17	23.28	27.76	21.98	5.22
Rotating Series	18.60	29.57	23.52	27.31	21.67	5.23
P - RD	3.46	3.80	-0.38	0.63	1.34	0.43
P - RS	4.50	5.26	1.34	2.14	2.57	0.36
Approx. standard error of differences	0.32	0.59	0.37	0.43	0.39	0.08

shallow. This result is probably not due to the mere increased depth of working, as the results of the ploughed plots showed that depth was not important. There is the possibility that the greater depth was in fact important for the particular kind of tilth produced by the rototiller and, as will be shown in the next section, by the grubber. A more probable explanation is that, because deep rototillage involved going over the land twice, it is due to the finer tilth and to the better burial of weeds and weed seeds on the deep than on the shallow tilled plots, both these differences being confirmed by Russell & Mehta's (1938) findings.

The deterioration of yield on the rototilled plots in the second over the first three-year period is given in Table 6. The table shows that the rate of deterioration of the barley and mangold yields appears to be independent of the cultivation treatment, except that the yield of mangolds on the continuously shallow rototilled plots appears to deteriorate rather slower than on the rest of the experiment.

Table 6. *The deterioration of yield on the rototilled plots*

	Wheat		Barley		Mangolds	
	(cwt./acre)	Grain	(cwt./acre)	Grain	Straw	(tons/acre)
Ploughed Continuous Series	2.20	7.63	11.92	13.65	7.41	-0.43
Continuous rototilled deep	5.53	10.65	11.70	13.90	6.55	-0.02
Continuous rototilled shallow	6.05	10.93	11.60	14.08	4.34	-0.08
Rotating rototilled: Deep	4.81	7.70	12.79	13.57	6.61	-0.29
Shallow	4.70	8.81	11.63	12.72	6.04	0.07

The results for wheat are in marked contrast. The rototilled plots have definitely deteriorated more than the ploughed plots, and as Table 7 shows this occurs on each of the four main treatments. This table also shows that the deep, and twice, rototilled plots of the Rotating

Table 7. *Decrease in yield of wheat grain in cwt./acre of the rototilled below the ploughed plots*

	Rotating Series		Continuous Series	
	Deep	Shallow	Deep	Shallow
1934-6	0.99	2.10	2.32	3.47
1937-9	4.95	5.03	6.25	6.72

Series yielded almost as well as the ploughed for the first three years. The beneficial effect of the deeper rototillage was not affected by the deterioration of yield, since compared with the shallow rototilled plots it produced mean increases of yield of 0.4 and 0.9 cwt. of grain per acre on the Continuous and 1.5 and 1.4 cwt./acre on the Rotating Series over the periods 1934-6 and 1937-9 respectively. Hence it is difficult to argue that the wheat yields are lower on the rototilled than on the ploughed plots because the tilth on the rototilled plots is too fine for winter wheat, as it is precisely those plots most likely to possess a deep fine loose tilth that give the smallest reduction of yield in the early years of the experiment.

COMPARISON OF THE PLOUGH AND GRUBBER

The mean yields of the crops over the six-year period are given in Table 8. The grubbed plots yield less in all cases than the ploughed, though the reduction for the deep grubbed is not very large for barley or mangolds. Again in all cases the shallow (and once) grubbed plots do not yield as well as the deep (and twice) grubbed ones. The continuously grubbed plots yield rather less than those grubbed in rotation though the difference is negligible for wheat and for mangold tops.

Table 8. *Mean yield of the ploughed and the grubbed plots*

	Wheat (cwt./acre)		Barley (cwt./acre)		Mangolds (tons/acre)	
	Grain	Straw	Grain	Straw	Roots	Tops
Mean ploughed, P	22.30	33.90	23.88	28.92	23.78	5.62
Grubbed: Deep, GD	18.81	29.04	22.67	27.40	21.90	5.22
Shallow, GS	17.74	28.07	21.83	26.53	21.06	5.31
Grubbed: Continuous Series	18.20	28.39	21.62	26.06	20.63	5.20
Rotating Series	18.35	28.72	22.87	27.87	22.13	5.34
P - GD	3.49	4.86	1.21	1.52	1.88	0.40
P - GS	4.46	5.83	2.05	2.39	2.72	0.31
Approx. standard error of differences	0.32	0.59	0.37	0.43	0.39	0.08

The deterioration of yield on the grubbed plots in the second three-year period compared with the first is given in Table 9. The deeper

Table 9. *Deterioration of yield on the grubbed plots*

	Wheat		Barley		Mangolds	
	(cwt./acre)		(cwt./acre)		(tons/acre)	
	Grain	Straw	Grain	Straw	Roots	Tops
Ploughed Continuous Series	2.20	7.63	11.92	13.65	7.41	-0.43
Continuously grubbed: Deep	6.57	11.98	12.83	13.48	7.56	-0.35
Shallow	6.33	9.65	10.52	12.60	5.26	-0.34
Grubbed Rotating Series: Deep	5.90	8.68	12.96	14.29	7.83	-0.42
Shallow	4.55	7.73	12.36	14.50	6.21	-0.45

grubbed plots seem to deteriorate rather more rapidly than the shallow, particularly on the Continuous Series, though this effect is only appreciable for wheat straw, barley grain and mangold roots. This is due to the level of yields on the deep grubbed plots of the Continuous Series having fallen to the level of the shallow grubbed plots of this series in the second three-year period, as is shown in Table 10. In the last three-

Table 10. *Decrease of yield of the Continuous shallow grubbed below the Continuous deep grubbed plots*

	Wheat		Barley		Mangolds	
	(cwt./acre)		(cwt./acre)		(tons/acre)	
	Grain	Straw	Grain	Straw	Roots	Tops
1934-6	1.27	2.63	2.08	1.70	2.27	-0.15
1937-9	1.02	0.30	-0.23	0.82	-0.03	-0.14

year period only wheat grain shows any benefit from deep as compared with shallow grubbing, and this is hardly significant as the three annual values of this benefit were 1.40, 2.40 and -0.75 cwt./acre. The mean yields for these three years were 9.5, 11.0 and 24.3 cwt./acre so that deep grubbing appeared to be of benefit when the yields were very low.

Table 9, in conjunction with Table 6, shows that the rates of deterioration of the barley and the mangold yields are the same for the plough, the rototiller and the grubber, so that this deterioration cannot be attributed to the method of cultivation. Presumably it is due to an inadequate supply of artificial fertilizers. Wheat, however, shows a greater deterioration on the grubbed than on the ploughed plots, as did the rototilled plots. When the results are analysed out in further detail, as in Table 11, it is seen that they show exactly the same behaviour as the rototilled, namely that in the first three years the wheat yield on the deep (and twice) grubbed plots was nearly the same as on the ploughed

plots but that in the second three-year period they had sunk to much lower values.

Table 11. *Decrease in the yield of wheat grain in cwt./acre of the grubbed below the ploughed plots*

	Rotating Series		Continuous Series	
	Deep	Shallow	Deep	Shallow
1934-6	0.91	2.21	1.40	3.43
1937-9	5.95	5.00	6.37	6.95

COMPARISON OF THE ROTOTILLED AND THE GRUBBED PLOTS

The mean differences in yield between the rototilled and the grubbed plots are given in Table 12. There is a general tendency for the grubbed plots to have a slightly lower yield than the rototilled, and this is still true when more detailed comparisons than those between full means are

Table 12. *Increased yield of the rototilled over the grubbed plots*

	Wheat (cwt./acre)		Barley (cwt./acre)		Mangolds (tons/acre)	
	Grain	Straw	Grain	Straw	Roots	Tops
1934-6	-0.24	0.81	1.03	0.48	-0.07	0.11
1937-9	0.33	0.82	1.27	0.66	0.76	-0.20
Mean	0.09	0.82	1.15	0.57	0.35	-0.04
Approx. standard error of mean	0.37	0.69	0.43	0.50	0.44	0.10

considered. For the first three years wheat grain, but not straw, did rather better on the grubbed than rototilled plots, particularly on the deep plots of the Continuous Series. Barley definitely did better on the rototilled plots, while the mangolds sometimes did better on the rototilled and sometimes on the grubbed. The main result of this comparison is, however, the smallness of the differences between these two cultivation treatments.

THE RESIDUAL EFFECTS OF THE ROTOTILLER AND GRUBBER

The possible existence of harmful residual effects on the crop yield due to using the rototiller or the grubber instead of the plough has already been mentioned in the section dealing with the crop yield on the ploughed plots. This point will now be examined in more detail. There were three different cultivation series in this experiment, the first containing those plots that always received the same method of cultivations, the Continuous Series, the second those plots in which the method of

cultivation followed the three-year cultivation rotation—plough-rototiller-grubber, called rotation A, and the third those plots cultivated in the order plough-grubber-rototiller—called rotation B. Thus the ploughed plots on the two Rotating Series differ from those on the Continuous Series in that the former were not ploughed in the two previous years while the latter were. If the grubbed or rototilled land was in a less favourable condition than the ploughed at the end of the cropping season, it is possible that it will carry a poorer crop in the following season if both are treated the same. Under these conditions the crop yield ought to be higher on the ploughed plots of the Continuous Series which have been ploughed all the time than on the ploughed plots of the Rotating Series, which were not ploughed the two previous years. Table 2 has already shown that grubbing and rototilling appear to have small harmful residual effects in comparison with the plough, but these differences are not precisely determined as they have been obtained from differences of yield between plots in different blocks and not between plots in the same block. A more detailed analysis of the data into two three-year periods does not give any additional confirmation of this result as the results of the comparisons become erratic.

A further method of examining the question whether grubbing or rototilling have harmful residual effects is to compare the mean yield of the rototilled plots of rotation A, which were ploughed the previous year, with those of rotation B which were grubbed the previous year and with those of the Continuous Series which were always rototilled. If the previous year's rototilling or grubbing left any harmful effects in the succeeding year that the ploughing did not, then the yield should be higher on rotation A than on rotation B or on the Continuous Series. In the same way the yield of the grubbed plots on rotation B which were ploughed the previous year should be higher than those on rotation A which were rototilled the previous year or on the Continuous Series

Table 13. *The harmful residual effects of not ploughing the land (method II)*

	Wheat grain (cwt./acre)		Barley grain (cwt./acre)		Mangold roots (tons/acre)	
	Rot. 1- Rot. 2	Rot. 1- Cont.	Rot. 1- Rot. 2	Rot. 1- Cont.	Rot. 1- Rot. 2	Rot. 1- Cont.
Rototiller:						
Deep	1.0	1.7	-0.1	0.1	1.9	0.2
Shallow	3.6 (a)	1.8 (a)	1.7	1.0	2.1	1.1
Grubber:						
Deep	-0.4	-0.4	-0.1	2.0	0.0	0.3
Shallow	-0.5	-0.1	2.1 (b)	3.3	0.0	3.1

which were always grubbed. The five-year means of these differences are given in Table 13. In this table rotation 1 is rotation A for the rototilled and rotation B for the grubbed series and rotation 2 is the other rotation. The wheat entries for the shallow rototiller series, marked (a) are almost entirely due to very high yields on the two plots of rotation A in 1937, which gave differences of 12.3 and 9.2 cwt./acre respectively for that year. The entry marked (b) for the barley yield under shallow grubbing is entirely due to very low yields on the two plots of rotation A in 1938, which gave a difference of 10.0 cwt./acre for that year. Cochran (1939) gave a rather more accurate method than this for estimating the residual effects of grubbing or rototilling the land, but the results of this more elaborate calculation do not differ appreciably from the simpler rotation 1-rotation 2 comparison.

The interpretation of Table 13 is not quite straightforward, since for the deep rototilled plots the difference rotation 1-rotation 2 really gives the residual effect of the shallow grubber compared with the shallow plough, for these are the treatments the plots received the previous year, while rotation 1-Continuous gives the residual effect of deep rototiller compared with the shallow plough. But the general impression one gets from the table is that the residual effects, if they exist, are small and very erratic.

There is still a third method of testing for any appreciable residual effects by comparing the difference of yield between all those ploughed and, say, those rototilled plots on the Rotating Series which were grubbed the previous year with the difference between the ploughed and rototilled plots on the Continuous Series. The first difference is what Cochran (1939) called the direct effect of the rototiller in comparison with the plough, since the previous year's cultivation was the same for each treatment and can be calculated rather more accurately than is described here. The second difference he called the continuous effect as it contains all harmful residuals resulting from rototilling the land year after year instead of ploughing it year after year. The difference between these represents the sum of any harmful residual effects due to the rototillings in the previous years.

Table 14 gives these harmful residual effects both for the full five years available and for the last three, when they should be most noticeable. This table again gives no evidence that there are any appreciable residual effects of not ploughing the seed-bed.

The conclusion reached is, therefore, that under the experimental conditions employed, there appeared to be no appreciable residual effects

Table 14. *The harmful residual effects of not ploughing the land (Method III)*

	Wheat grain (cwt./acre)		Barley grain (cwt./acre)		Mangold roots (tons/acre)	
	1935-9	1937-9	1935-9	1937-9	1935-9	1937-9
Plough minus rototiller:						
Deep	1.5	0.4	0.1	-1.0	-0.5	-1.7
Shallow	0.6	0.3	0.9	1.2	0.6	0.3
Plough minus grubber:						
Deep	-0.8	-0.8	1.4	0.4	0.7	-0.7
Shallow	0.3	0.5	2.1	0.9	2.9	2.2

of grubbing or rototilling the land instead of ploughing it in the following year. But this conclusion is limited by the important experimental condition that all the plots were shallow ploughed as soon after the wheat was harvested as possible in preparation for the mangold crop.

MISCELLANEOUS OBSERVATIONS ON THE CROP GROWTH

A number of eye observations have been taken on the crop, mainly at harvest. The usual remarks for the wheat and barley are that the ploughed plots carry a taller plant ripening more evenly than the cultivated plots. The shallow cultivated plots usually carried a gappy plant and in a bad year one of very variable height and for barley containing many green ears at harvest. In general the ploughed plots carried the fewest and the shallow cultivated plots carried the most weeds. There was, however, no close connexion between total yield and general weediness during the growing season, since the deep cultivated plots sometimes gave the same crop yield but carried a larger weed population than the ploughed.

There were exceptions to these generalizations. Two blocks of the barley experiment in 1938 provide an example when they did not hold. There was a poor early stand on the ploughed and the grubbed plots and a better, but still only mediocre, stand on the rototilled plots. This was not due to weeds as the two blocks were still clean but may have been due either to differential bird damage or to poor tilth causing uneven germination. There were indications that the bare patches had a rougher tilth and were a little drier than where the barley was showing, but the differences were only small. At harvest all eight of the rototilled plots were described as having an even plant of uniformly ripe ears while only one of the eight ploughed plots was so described and none of the grubbed, though one had uniformly ripe ears. None of the rototilled

plots were described as weedy though several of the ploughed and grubbed were. In this case weediness was a result of the patchiness of the crop and not a cause, whereas normally it is at least a partial cause.

One other observation was made on the wheat crop in four out of the six years of the experiment. The wheat ears tended to be larger on the deep ploughed plots than on the shallow but the number of ears rather fewer. In three of these years the straw was noted as being taller and stronger on the deep ploughed than the shallow ploughed plots. These two effects were also noted on the deep grubbed plots in 1935.

Weeds were usually more in evidence in the early stages of growth on the non-ploughed mangold plots, particularly on those shallow tilled, though they could never dominate the crop for long as it was hoed at least once before and usually several times after singling. At the time of pulling all the plots were clean, the only exception being in 1936 when half the plots could not be properly hoed towards the end of the season with the consequence that they carried a strong weed population throughout the latter part of the growing season. These late weeds did not, however, affect the yield.

The number of mangold roots pulled per plot was counted every year, and except for 1935 was almost independent of cultivation treatment as is shown in Table 15. In 1935 the effect of treatment is marked,

Table 15. *Mean number of mangold roots harvested per plot**

(1934-9, omitting 1935.)

	Plough	Rototiller	Grubber
Deep	162	161	159
Shallow	164	159	160

* To obtain roots per acre multiply by 130.

Table 16. *Mean number of mangold roots harvested per plot in 1935*

	Continuous Series			Rotating Series		
	Plough	Rototiller	Grubber	Plough	Rototiller	Grubber
Deep	168	157	157	166	159	146
Shallow	181	140	103	173	149	155

as is shown in Table 16. The plant numbers were highest on the ploughed plots, and in the Continuous Series there was a considerable reduction on the shallow rototilled and a great reduction on the shallow grubbed plots. This result is probably due to the great variations in the weediness of the seed-beds produced by the various cultivation implements. Russell & Mehta (1938) showed that in 1934 there was a poorer apparent

germination of mangolds on the weedier plots than on the cleaner, but that it was sufficient on every plot for an almost even plant to be set at singling. In 1935 the seed-beds on the non-ploughed plots were weedy, and the shallow grubbed plots of the Continuous Series were particularly foul. Weeds seem to have caused a sufficiently large mortality among the young seedlings on some of these plots to prevent a full plant being set, and hence the large variations in plant number. To overcome this trouble in future years it was decided to plough the whole of the wheat stubble to a 4 in. depth in the autumn of 1935 and every subsequent year in preparation for the mangold seed-beds. This had the desired cleaning effect so that in the subsequent years a fairly even plant was set at singling as can be seen from Table 15.

The roots were sometimes rather smaller on the rototilled and the grubbed plots than on the ploughed, but the differences were not large, as is shown in Table 17.

Table 17. *Mean root weight of mangolds in lb.*

	Plough	Rototiller	Grubber
1934-6: Deep	2.96	2.91	2.85
	2.75	2.76	2.83
	2.86	2.83	2.84
1937-9: Deep	2.19.	1.96	1.93
	2.06	1.95	1.91
	2.13	1.96	1.92

One other comment was made about the mangolds in each of the last three years. At lifting time the leaves were predominantly dark green on the deep ploughed plots, a lighter green on the shallow ploughed becoming yellowish or yellow on the rototilled and the grubbed, this being more marked on those shallow than those deep tilled.

THE RELATIVE INFLUENCE OF SEED-BED TILTH AND WEEDS ON CROP YIELD

The question that arises from these experiments is how far the differences in crop development on the various plots are due to differences in the tilth of the seed-bed at the time of sowing and how far to differences in weed infestation. No definite answers can be given to this question since direct estimates of seed-bed tilth and weediness during the growing season are not available. There is no question that the very low yields on some plots was due to them being foul with weeds, but the question of greater importance is whether the average losses of crop due

to grubbing or rototilling are due to the greater weed population they carry or to their giving a seed-bed having a poorer tilth.

There is one general consideration pointing to weeds being an important factor in causing this reduction in yield. The rototiller working shallow always seemed to prepare as good a seed-bed as the shallow plough treatment, yet the yields were usually lower and sometimes considerably lower while the weed infestations were often considerably higher. The rototiller working deep gave a deeper seed-bed which was probably only a little looser and a little finer than when working shallow, but it usually carried a larger crop with fewer weeds. Since the depth of ploughing did not affect the yield, it seems quite plausible to assume that it was the decreased weediness rather than the increased depth of seed-bed that was mainly responsible for the increased yield.

Turning to the crops individually, it was often noted that the wheat straw was rather shorter on the non-ploughed than on the ploughed plots. It is unlikely this would be a seed-bed effect as the crop is winter sown, but is much more likely to be due to weed competition for available nitrogen as suggested by Blackman & Templeman (1939), particularly since this was most noticeable at the end of the experiment when deterioration of yield due to insufficient manuring was probably setting in.

A second interesting point is that there was a definite tendency for the grain on the deep ploughed plots to be plumper and the straw taller but for the crop to be less uniform than on the shallow ploughed, so that the yield per acre was unaffected. It is possible that the plumper grain and the taller straw may have been due to the slightly greater freedom from weeds and that the greater unevenness of plant was due to the rather rougher and less uniform seed-bed on the deep than on the shallow ploughed plots.

There is a general belief that barley starts off much better if it has a fine seed-bed; this may be the reason why the deep rototilled plots gave as good a yield as the ploughed, although they carried a heavier weed population. Here tilth is almost certainly of some importance and this is probably borne out by the 1938 barley results already discussed. The evidence for the harmful effect of weeds is less clear than for wheat. In the first place the mean reduction of yield on the shallow grubbed plots, usually the weediest, below the ploughed plots is only 2 cwt./acre for the barley grain compared with 4½ cwt./acre for the lighter wheat crop. But the barley followed the mangolds so weed competition may have been less severe.

Weeds early in the season, however, might easily set the plant back somewhat, thus making it less uniform in height and maturity at harvest than the cleaner plots, as observed. But if this is the correct explanation one would expect it to be due to competition between the weeds and the barley for the nutrients and in particular for the available nitrogen. Now the barley crop showed very marked deterioration in yield in the second three-year period, but this deterioration has been shown to be the same on the comparatively clean ploughed plots as on the dirty grubbed ones, and is six or seven times as large as the loss of yield due to using the grubber. The competition between the weeds and the barley for nutrients must, therefore, have been very small if it were the cause of lowering of yield of the grubbed compared with the ploughed plots.

The mangold crop appears to be more affected by the weediness than by the tilth of the seed-bed, though probably in each year the variations in tilth from plot to plot were considerably smaller than the variations in weediness. Weeds compete very successfully with the young mangold plants in the first stages of their growth either by killing them off or by giving them a set-back from which they never completely recover. This is the probable cause of the harvest observation that the mangold leaves go yellow soonest on the shallow non-ploughed plots and keep greenest longest on the deep ploughed plots for this is the order of seriousness of weed competition in the seed-bed. This argument is barely invalidated, as it seems to be for the barley crop, by the result that the rate of deterioration of yield is the same on the ploughed and the non-ploughed plots, for after the first 2-3 weeks of crop growth all the mangold plots are kept equally clean by hoeing for the rest of the growing season. There is only a greater shortage of nutrients on the unploughed than on the ploughed plots for the very early and not for the greater part of the growing season but, as Brenchley (1929) has shown, the future physiological development of the crop is especially sensitive to shortage just at this time.

Thus while no definite conclusions can be drawn from the experimental data on whether less suitable tilth or increased weediness is the cause of the crops on some cultivation treatments yielding less than on others, the generalization that winter wheat and mangolds are more susceptible to weeds and less to seed-bed tilth than barley appears to be consistent with the experimental results.

THE PRACTICAL CONCLUSIONS FROM THESE RESULTS

The main result that has emerged from this experiment is the very great importance of good weed control in the seed-bed. The great virtue of preparing a seed-bed by ploughing and then breaking up the furrow with harrows and rolls is that by turning the furrow slice right over, perennial and annual weeds are kept in check while the young crop is germinating and beginning to grow. Good tilth by itself does not ensure this condition, and in fact this experiment gave the impression that the development of the crop was not very sensitive to variations in the tilth of the seed-bed provided it was clean.

This result, which is in accord with British agricultural practice, means that the plough, by turning over the furrow slice can be a most effective controller of weeds in the early stages of the crop development, and no method of seed-bed preparation that does not ensure this burying of weeds and weed seeds can be of more than limited use.

But this result also means that if the land is already clean, as it often is after a root crop, any method that gets a reasonable tilth quickly can be used without the necessity for a ploughing.

The second general conclusion is that except possibly for the mangold crop the depth of working the seed-bed appeared to be of no importance, since there was no appreciable difference in yield between plots ploughed to a 4 in. or to an 8 in. depth for the whole of the six-year period, the largest difference being about 5 % for the mangold crop. This must not be taken to mean that ploughing below 4 in. is never beneficial, but it does mean that, as far as crop yield at Rothamsted is concerned, if the soil has been ploughed to below 4 in., it is probably unnecessary to repeat this depth for at least six years. Ploughing to 8 in. did, however, appear to depress the weed population rather better than ploughing to only 4 in., and this beneficial action is probably more important the more weedy the land being ploughed.

A corollary follows from these two conclusions, namely that the higher yield usually obtained by going over the land twice with either the rototiller or the grubber to get an 8 in. depth of working rather than only once to get a 4 in. depth was almost certainly due to the better weed control obtained. Hence if the land is not quite clean it is still unnecessary to plough it provided the cultivators go over the land more than once so that they thoroughly disturb all the weeds present.

SUMMARY

1. The results of a six-year cultivation rotation experiment are given. The rotation used was wheat-mangolds-barley and the seed-beds for these were prepared either by ploughing, using a rotary cultivator or a tractor-drawn grubber.

2. The yields of these crops were barely influenced by the depth of ploughing, a 4 in. depth giving throughout the six years just about the same yield as an 8 in. depth. The mangold crop was possibly a little larger on the deeper ploughed plots.

3. The mean yields of the seed-beds prepared with the tractor drawn grubber or cultivator followed by harrows etc. were lower than the ploughed seed-beds for all the crops, and this was particularly so on those seed-beds prepared by only one grubbing down to 4 in. depth.

4. The mean yields on the seed-beds prepared by the rototiller were lower than on the ploughed seed-beds for wheat and mangolds. If the seed-bed was prepared by rototillage to a depth of 8 in. by going over the land twice, the yield of barley was the same as on the ploughed seed-beds, but was definitely less on the seed-bed rototilled only once to 4 in.

5. Seed-beds prepared by the rototiller or grubber have only a small residual effect on the crop yields in the following year.

6. It is concluded that the primary function of ploughing is weed control, and that it is only advisable to omit ploughing either if the land is already fairly clean or if the crop will be hoed very early on in its development.

7. For wheat and mangolds differences in weed infestation of the seed-bed were probably of greater importance than differences in tilth in so far as the crop yield was concerned. The reverse may have been true for barley.

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FURTHER INVESTIGATIONS ON ARTIFICIAL INSEMINATION OF CATTLE

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A PRELIMINARY investigation of artificial insemination of cattle in Kenya was made during 1936 and part of 1937 (Anderson, 1938). In this paper experiments up to August 1939 are described. The earlier results are repeated here for comparison and also because the results from farms 1 and 2 in the previous report were based on cows 'holding' and not on actual calvings.

MATERIAL AND METHODS

The technique used, which is similar to that of Walton (1936) and Russian workers, has already been fully described (Anderson, 1937, 1938). The bulls which provided the sperm were mostly pure-bred bulls and the cows were all high-grade cows, except for one experiment with Zebu cows. In the Experimental Station herd the type was predominantly Shorthorn; on farm 1, Friesian; and on farm 2, Ayrshire. All the results in the paper are based on calvings.

RESULTS

The results of the experimental investigation of artificial insemination on three farms are shown in Table 1.

Table 1

Farm	No. of cows	Calved		No. of inseminations	No. of inseminations	
		No.	%		Per conception	Per cow
E.S.	451	385	85.4	662	1.72	1.47
1	185	108	58.4	273	2.53	1.48
2	97	70	73.7	123	1.76	1.29
Total	733	563	76.8	1058	1.88	1.44

The herds on farms 1 and 2, particularly that on farm 1, were affected by the contagious venereal disease peculiar to Kenya, which is associated with vaginitis in the cow and epididymitis in the bull (Daubney *et al.* 1938; Anderson, 1939, 1940). It is believed that the prevalence of this

disease may have been responsible for the low percentage of calvings on farm 1. Artificial insemination has been practised on this farm since the end of the above experiment in March 1937, by the Manager, and the calvings are said to be satisfactory. On farm 2, subsequent to the above experimental period, a further twelve cows calved to artificial insemination giving a calving percentage of 86.3.

The Experimental Station herd has never been affected by venereal disease, and since 1935 it has been free from contagious abortion. The annual percentage of cows calving to artificial insemination in this herd has been satisfactory (Table 2). 80% fertility was obtained in this herd in the period 1929-36 from ordinary service.

Table 2

Year	No. of cows	Calved	
		No.	%
1936	83	69	83.1
1937	114	104	91.5
1938	133	117	88.0
1939	121	95	78.6

The insemination records of sixteen bulls are shown in Table 3. The number of inseminations required for conception was similar on the Experimental Station and farm 2; on farm 1 the number required was higher because of genital disease in the cows.

Table 3

Farm	Bull	Breed	No. of inseminations	No. of conceptions	No. of inseminations
					per conception
E.S.	S 1	Shorthorn grade	173	96	1.80
	Ag	Ayrshire grade	40	18	2.22
	A 2	Ayrshire	42	19	2.21
	A 3	Ayrshire	19	7	2.71
	H	Hereford	92	61	1.51
	AA	Aberdeen-Angus	71	45	1.58
	Su	Sussex	45	18	2.50
	F	Friesian	20	17	1.18
Total				281	1.79
1	A	Friesian	94	43	2.19
	B	Friesian	104	43	2.42
	C	Friesian	75	22	3.41
	Total			108	2.53
2	A	Ayrshire	28	19	1.32
	B	Ayrshire	30	20	1.33
	C	Ayrshire	40	23	1.74
	D	Ayrshire	17	6	2.83
	E	Ayrshire	8	2	4.00
	Total			70	1.76

Table 4

	E.S.		Farm 1		Farm 2		
	No.	%	No.	%	No.	%	
Cows calved to:	1st insemination	241	67.1	95	73.1	54	77.2
	2nd insemination	76	21.2	24	18.4	14	20.0
	3rd insemination	23	6.4	9	7.0	2	2.8
	4th insemination	11	3.1	2	1.5	—	—
	5th-7th insemination	8	2.2	—	—	—	—

From 2 September 1937 to 1 December 1937, 118 Zebu cows were artificially inseminated, and 174 inseminations were performed, i.e. 1.47 per cow using Experimental Station bulls. Seventy cows calved (59.3%), giving a ratio of 2.43 inseminations per conception.

On farm 1 there was no significant difference in the number of inseminations required for conception, using a dose of 1 ml. undiluted sperm, 1 ml. sperm diluted $\times 2$, and 1 ml. sperm diluted $\times 4$. An average of 2.5 inseminations per conception were required on this farm which is high compared with the two other herds. On farm 2 there was also no significant difference in the number of inseminations required for conception. In the Experimental Station herd, on the other hand, there were highly significant differences between the different experiments ($\chi^2 = 19.6$ for 3 degrees of freedom, which exceeds the 1% point). The number of inseminations required for conception varied from 1.30 with

Table 5

Procedure	Farm	No. of cows	No. of inseminations	Calved		No. of inseminations	
				No.	%	Per conception	Per cow
Single insemination							
1 ml. undil.	E.S.	181	260	127	70.2	2.05	1.44
	1	28	42	17	60.7	2.24	1.50
	2	76	91	52	68.4	1.75	1.20
	Total	285	393	196	68.8	2.01	1.38
0.5 ml. undil.	E.S.	67	73	56	83.4	1.30	1.09
0.5 ml. dil. $\times 2$	E.S.	70	74	42	60.0	1.76	1.06
1.0 ml. dil. $\times 2$	E.S.	68	91	52	76.5	1.75	1.49
	1	87	103	40	46.0	2.58	1.17
	2	30	32	18	60.0	1.78	1.07
	Total	185	226	110	59.5	2.05	1.22
1.0 ml. dil. $\times 4$	1	97	128	51	52.5	2.51	1.32
Double insemination							
0.5 ml. undil.	E.S.	56	56	38	67.9	—	—
1.0 ml. undil.	E.S.	79	100	62	78.5	—	—

a dose of 0.5 ml. undiluted sperm to 2.05 with a dose of 1.0 ml. sperm also undiluted.

But, although these differences were significant in the Experimental Station herd for the period 1936-9 they were not confirmed, when with the object of eliminating any possible seasonal effects on fertility, the experiments were repeated during September 1938 to March 1939 (Table 6).

Table 6

Procedure	Period	No. of insemina- tions	Calved		No. of in- seminations per cow
			No. of cows	No. %	
1 ml. undil.	19. xi. 38-9. iii. 39	42	29	69.0	1.48
0.5 ml. undil.	5. ix. 38-27. i. 39	27	19	70.4	1.43
0.5 ml. \times 2 undil.	30. i. 39-17. iii. 39	21	11	57.1	2.00

$$\chi^2 = 1.88.$$

Two inseminations in the one heat period did not give better results than a single insemination. 69% of forty-two cows which received 1 ml. of undiluted sperm calved (Table 6), compared with 68% of fifty-six cows which received two inseminations of 0.5 ml. sperm in the one heat period (Table 5).

DISCUSSION

It is believed that when highly fertile bulls are used with normal, healthy cows about 1.5-2.0 services are required per conception (Anderson, 1938, 1939). The figures obtained from artificial insemination in different countries mostly fall within this range. Davis (1939), for example, found a variation of from one to four inseminations per conception for different bulls with an average of 1.69, and Henderson's (1939) figures for individual bulls varied from 1.39 to 2.69 inseminations per conception with an average of 1.91. There was considerable variation in the number of inseminations required for conception in the Experimental Station herd and on farms 1 and 2, but the mean figures for the Experimental Station and farm 2 were within the normal range. On farm 2 the figure was raised by genital infection.

On the basis of 1.5-2.0 services per conception, from 50 to 67% of cows should conceive to a single insemination. In Sorenson's (1938) 1936-7 experiment, of 1157 cows, 555 conceived to the first insemination, i.e. 49%, a figure similar to that obtained by Bell (1938) whose cows were inseminated once only and 51.3% conceived; 67% had conceived by the second insemination, 75.9% by the third and 78.8% by the fourth. Bonadonna (1939) stated that pregnancy was obtained in

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60-80% of cases with a single insemination, and up to 90% with one, two or three inseminations. In the Experimental Station herd about 58% of the inseminations were fertile.

Of cows that conceived, Sorenson found that 59.2% did so at the first insemination. Davis found that 66.7% resulted from one insemination, 21.5% from two inseminations, 7.5% from three, 8.9% from four and 1.9% from five inseminations. Very similar results were obtained in the Experimental Station herd (Table 4).

To obviate different levels of fertility in the three herds it is necessary to compare experiments in each herd. On farms 1 and 2 there was no significant difference between the use of undiluted sperm and sperm diluted up to a maximum of $\times 4$ with dilutor GTC. In the Experimental Station herd there was a significant difference between experiments using different doses and dilutions of sperm over the whole period reported in this paper. These experiments, however, were carried out under different conditions, at different times of the year, using sperm from different bulls. The results are not therefore solely attributable to dilution and dose of sperm. In fact, when the experiments were carried out at much the same period of the year no significant differences were found between using 1 ml. of undiluted sperm, 0.5 ml. of undiluted sperm, and 0.5 ml. of sperm diluted $\times 2$.

Russian workers (Andreev, 1937; Kirillov, 1937) found that two inseminations during a single heat period gave better results than a single insemination. This observation does not apply to grade cows in Kenya, perhaps because of the relatively short period of heat experienced by these cows.

SUMMARY

An account is given of artificial insemination of 733 grade cows in Kenya from January 1936 to August 1939. 76.8% of the cows inseminated calved, with an average of 1.88 inseminations per conception. The different doses and dilution of sperm used gave very similar results, as did two inseminations, compared with one insemination, in a single heat period.

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FURTHER INVESTIGATIONS ON ARTIFICIAL INSEMINATION OF SHEEP

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PRELIMINARY experiments on artificial insemination of high-grade Merino sheep in Kenya were made in 1935-6 (Anderson, 1937). Further experiments made from 1936 to 1939 are reported in this paper.

As far as the author is aware, very little work has been done on artificial insemination of Merino sheep. A small number of ewes have been artificially inseminated in Australia (Gunn, 1936; Kelley, 1937) and in South Africa (Quinlan *et al.*, 1936). Gunn inseminated 136 Merino ewes and thirteen lambred. The procedure adopted, however, was not such as would give the best results, e.g. in some cases the sperm was collected from the vagina of a ewe; physiological saline solution was used for diluting sperm; and in some cases, sperm was kept for as long as 6 hr. at room temperature before use. Kelley (1937) obtained 22% of lambs from artificial insemination of fifty-five ewes. In the first experiment made by Quinlan *et al.* in South Africa, sperm was collected from the vagina of a ewe and 1-2 ml. sperm was diluted with 7-8 ml. Tyrode solution, 1 ml. of which was introduced into the vagina. Of twenty-five ewes inseminated with fresh diluted sperm fourteen (56%) lambred. In the second experiment an artificial vagina was used for collecting the sperm. The sperm was diluted 1 in 3 with GPS-8, and inseminations were carried out within $\frac{1}{2}$ hr. of collecting the sperm. A single insemination was made into the cervix at one heat period. Of forty ewes inseminated nineteen (47.5%) lambred. There was a difference of 10% in favour of hand-service. These workers suggest that driving of sheep, which causes a rise in temperature, before insemination, may have reduced the percentage of pregnancies. These results, however, are very similar to those which have been obtained at Naivasha, and it is probable that the low percentage of conceptions is related, partly at least, to the low fertility of Merino sheep.

Quinlan and co-workers state that the technique of artificial insemination is so complicated that it is unlikely to be of economic value to the rural population of South Africa in the normal breeding of domestic

animals, but more intensive use may be made of exceptional sires in large stud flocks and the services of an experienced technician will be warranted. The author does not agree with this view as far as Kenya is concerned. The technique of artificial insemination is not so complicated that it cannot easily be put into practice by the average person after a short period of training. The results obtained experimentally in Kenya have shown that artificial insemination can be successfully applied to sheep in Kenya, and good results have also been obtained by those farmers who have themselves used the method.

MATERIAL AND METHODS

The rams used were all pure-bred Merinos and the ewes were high-grade Merinos, which have been bred up in Kenya for many generations by crossing a local fat-tailed type of ewe with imported Merino rams.

Vasectomized rams were used to pick out ewes on heat. Details about the time of insemination during oestrus, the number of inseminations during each oestrous period, the degree of dilution of sperm, and the dose of sperm are given in the appropriate places, later in the paper. The technique used, which is similar to that of Walton (1936) and Russian workers has already been described (Anderson, 1937, 1938).

RESULTS

The total results obtained from artificial insemination of sheep at the Experimental Station, Naivasha, for the period 1935-9 are shown in Table 1.

Table 1

Period	Farm	No. of ewes	Ewes lambed	
			No.	%
1935-9	E.S.	1817	1183	65.1
1936, 1937	W	4203	2106	50.1

The results from 'E.S.' (Experimental Station) ewes were better than from the 'W' ewes, but they are both below what is normally obtained from ordinary service. Results comparable with those from ordinary service have however, been obtained from certain experiments, and it is now known that the procedure adopted in some of the experiments was not such as would give the best results, as is explained later (Table 2).

No attempt was made to inseminate the maximum number of ewes with one ram. Nevertheless, the number of rams actually used was

Table 2

	1935-6	1936	1937	1938	1939	1935-9
Av. no. of ewes inseminated by 1 ram	202	652	456	131	300	348
Av. no. of lambs born to 1 ram	150	201	297	61	245	191

considerably less than formerly, when ordinary service was practised. Previous to 1935, eighteen rams were kept for a flock of about 600 ewes, and of these rams eleven were pure-bred Merinos and seven were grade Merinos. From 1935 to 1939 an average of three rams was used per breeding season. The number of ewes inseminated on an average by one ram and the number of lambs born on an average to one ram as the result of artificial insemination thus showed a considerable increase over that obtained from ordinary service. In the period 1935-9 an average of 148 ewes were inseminated by one ram and an average of 191 lambs were born to one ram. In 1937 one ram sired 411 lambs.

Season. The results obtained with the Experimental Station ewes at different periods of the year are given in Table 3. The better results

Table 3

Period of insemination	No. of ewes inseminated	% of ewes lambed	Experimental ewes	
			Length of oestrous cycle in days	% of ewes on heat
April-June, 1937	462	79	19.3	100
April-July, 1939	601	75	17.3	92
Dec. 1935-Feb. 1936	202	74	18.6	—
June-Aug. 1938	393	47	24.6	87
June-Aug. 1936	159	25	29.2	66

compare favourably with the normal lambing from such sheep after ordinary service. On two occasions, however, the results have been unsatisfactory. It is of interest to compare the results from artificial insemination with the length of the oestrous cycle and the incidence of oestrus in experimental ewes (author, unpublished results) for the same periods of the year (Table 3). There is a very distinct correlation between the percentage of ewes that lambed and the length of the oestrous cycle and the percentage of ewes on heat. The longer the cycle and the smaller the percentage of ewes on heat, the poorer was the lambing. Better results were obtained from artificial insemination at those periods of the year when the oestrous cycle was short and the incidence of oestrus was high. The main factor in obtaining a satisfactory lambing was therefore the season of the year at which artificial insemination was practised.

It was observed in 1938 that considerable variation occurred in the incidence of lambing. 166 ewes were inseminated twice in the one oestrous period from 12 July to 21 August, omitting the period from 9 to 15 August (Table 4). 69.3% of these 166 ewes came on heat in

Table 4

Period	No. of ewes	% of total ewes on heat	% of ewes lambed
12-18 July	31	18.7	12.9
19-25 July	58	34.9	36.2
26 July-1 Aug.	26	15.7	42.3
2-7 Aug.	37	22.3	16.2
17-21 Aug.	14	8.4	50.0

20 days and 91.4% in 27 days. It seems that conditions were especially favourable for conception during certain short periods in the course of this experiment.

Dilution of sperm. Sperm used undiluted, and diluted up to a maximum of $\times 8$ has proved equally effective; beyond this point there was a marked reduction in the percentage of conceptions (Table 5). All experiments using a dilution of $\times 8$ have not, however, given similar results; the percentage of conceptions in different experiments varied from 20 to 54.

Table 5

	Dose 0.2 ml. sperm undiluted	Sperm diluted				Dose 0.1 ml. $\times 8$
		$\times 3$	$\times 4$	$\times 8$	$\times 16$	
No. of ewes	47	50	65	925	669	533
No. of ewes lambed	22	21	29	412	115	81
% of ewes lambed	46.8	42.0	44.6	44.5	17.2	15.1

Cooling of sperm. The cooling of sperm had little effect on the conception rate (Table 6).

Table 6

	No. of ewes	% lambed
Sperm cooled to 10° C. and used cool	47	17.0
Sperm cooled to 10° C. and warmed by hand before use	62	17.8
Sperm used uncooled	57	22.0

Number of inseminations. Up to 1937 season the ewes were inseminated once during each heat period and thereafter twice or more (twice unless otherwise stated). The comparison between 1937 and 1939 is shown in Table 7.

In 1937 the ewes were picked out in the morning and inseminated once only in the morning. In 1939 the ewes were picked out in the

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Table 7

No. of oestrous periods ...	1st	2nd	3rd	4th	5th
April-June, 1937					
No. of ewes	1516	1171	782	483	136
% of ewes lambed	19.5	21.3	23.5	22.6	27.9
June-August, 1939					
No. of ewes	601	408	288	81	—
% of ewes lambed	27.6	29.3	32.6	25.9	—

morning, and during the day up to about 3 p.m. and these ewes were inseminated twice, once that evening and again the following morning. The results from this latter procedure were better than from the former.

Table 8

June-August, 1938

	No. of ewes	% lambed
Single insemination (a)	27	14.8
Single insemination (b)	29	3.5
Single insemination (c)	29	6.9
Double insemination (d)	166	29.5

(a) Ewes picked out a.m. and inseminated that p.m.

(b) Ewes picked out a.m. and inseminated the following a.m.

(c) Ewes picked out a.m. and inseminated the following p.m.

(d) Ewes picked out a.m. and inseminated that p.m. and following a.m.

Results very similar to those obtained in 1939 were obtained from a double insemination in 1938. Single inseminations at different times in relation to oestrus gave poor results (Table 8).

Table 9. *One or more inseminations in the one heat period*

No. of inseminations	No. of ewes	% lambed
1	5	0
2	56	37.5
3	16	37.5
4	19	48.4
Total	96	37.5

In Table 9 is shown the results from inseminating ewes, once, twice, three times and four times, according to the length of oestrus. The ewes were picked out in the morning and inseminated; if still on heat that evening they were again inseminated; if still on heat the following morning they were inseminated a third time and so on. This experiment, which lasted for 14 days from 25 November to 8 December 1937, gave a 37.5% lambing. One ram was used for all the ewes. However, on occasion a single insemination has given equally as good or better results

as two or more inseminations in the one heat period, as for example in 1935-6 (Anderson, 1937), and in the experiments detailed above using sperm undiluted and diluted up to $\times 8$. It is probable that this fact is related to the period of the year at which the inseminations were carried out.

Ordinary service in August 1936 of 309 ewes (the ewes were run with rams for 3 weeks) gave a 74% lambing and of 96 ewes (hand service) gave 70% lambing (author, unpublished results). There was thus little difference between a single service and several services, but the results from a single service are better on the average than the results from a single insemination. Artificial insemination in June-August 1936, using a single insemination in each heat period, gave poor results (25% lambing) compared with a single service per heat period (70% lambing).

The percentage of fertile inseminations for different rams in 1936 and 1937 is shown below. The results are not strictly comparable since the inseminations were not all made at the same period and different dilutions, some of which were too high, were used.

Table 10

Rams	1936			1937	
	57	708	3237	3062	3068
No. of inseminations	344	77	67	1993	732
No. of inseminations fertile	150	8	13	453	139
% of inseminations fertile	43.6	10.4	19.3	22.7	19.0

Time of insemination in relation to oestrus. Two experiments, involving 242 ewes, were carried out in 1938 and 1939, to determine the conception rate when ewes were inseminated at different intervals from the beginning and end of oestrus. The results are shown in Table 11.

The actual conception rates in the two experiments are not strictly comparable since the ewes were inseminated at different periods of the year in 1938 and 1939 and different rams were used. The experiments show that the period from 12 to 30 hr. after the onset of oestrus was the most favourable to conception. During this period in 1938, 25% of the ewes conceived, and in 1939, 24% conceived.

The duration of oestrus in the inseminated ewes in 1938 was 20.8 hr. The duration of oestrus is not known for the inseminated ewes in 1939, but for other experimental oestrous cycle ewes at the same period of the year it was 28.6 hr. There is a minimum period of about 26 hr. before which ovulation does not occur in grade Merino ewes in Kenya (Anderson, 1938b), and when oestrus lasts longer than about 26 hr. ovulation occurs

Table 11

Period of oestrus at which ewes inseminated hr. from onset of heat	1938		1939	
	No. of ewes	% lambed	No. of ewes	% lambed
1-6	20	25.0	25	0
12-18	28	42.9	25	20
24-30	32	9.4	25	24
36-42	29	0	25	12
48-54	32	0	—	—

Duration of oestrus in hr.				
No. of ewes	No. lambed	Mean	Standard deviation	Range
Insemination 1-13 hr. after the end of oestrus				
27	12	14.8 ± 0.67	3.5	10.0-22.0
18	1	18.2 ± 1.01	4.4	8.2-26.6
9	0	31.5	—	29.6-36.0
Insemination 14-39 hr. after the end of oestrus				
50	0	17.9 ± 0.29	6.09	6.8-30.1

very shortly after the end of oestrus. The longer period of oestrus in the 1939 experiment therefore probably explains the somewhat later interval after the onset of oestrus at which most conceptions occurred.

Insemination after the end of oestrus is effective when the length of oestrus is short, and within the limits of the experiment, the shorter the average duration of oestrus, the higher was the conception rate, when insemination was performed 1-13 hr. after the end of oestrus. No conceptions occurred when insemination was carried out 14-39 hr. after the end of oestrus, which lasted on the average for 18 hr.

For the thirteen ewes which lambred from insemination 1-13 hr. after the end of oestrus, the mean duration of oestrus was 14.7 hr.; the interval between the end of oestrus and insemination was 8.3 hr.; and the interval between the onset of oestrus and insemination was 23 hr. These results must be considered in relation to the time of ovulation. In the thirteen ewes with short oestrous periods, which were inseminated 1-13 hr. after the end of oestrus, the sperm was introduced a few hours before the probable time of ovulation. In the nine ewes whose mean length of oestrus was 31.5 hr. and which were inseminated 1-13 hr. after the end of oestrus, and in the fifty ewes whose mean length of oestrus was 17.9 hr. and which were inseminated 14-39 hr., the sperm was introduced after the probable time of ovulation.

Thirty-five ewes were inseminated 8-23 hr. before the end of oestrus and six lambred. The mean length of oestrus in the six ewes that lambred was 23.2 hr. and the mean interval between insemination and the end

of oestrus was 14.7 hr. Of nineteen ewes inseminated up to 19 hr. before the end of oestrus two lambred; of eight ewes inseminated 13-18 hr. before the end of oestrus two lambred; and of eight ewes inseminated 19-22.5 hr. before the end of oestrus two lambred. Ram sperm thus remained alive and fertile in the genital tract of the ewe for 22.5 hr., the approximate time between insemination and ovulation.

DISCUSSION

Season. It is well known that Merinos in certain parts of Australia and South Africa, when conditions are favourable, experience a continuous series of dioestrous cycles throughout the year (Marshall, 1922; Quinlan & Mare, 1931). According to Quinlan & Mare (1931) this occurs under Great Karroo conditions in South Africa. Kupfer (1929), on the other hand, observed the existence, under western Free State conditions in South Africa, of a prolonged anoestrous period in Merino ewes. Roux's (1936) observations agree to a very great extent with those of Kupfer. In Australia, Kelley & Shaw (1939) have shown that Merino ewes have a well-defined periodicity in the percentage of ewes coming on heat. There was a fall in the incidence of oestrus in the spring months, followed by a rise in the summer months, the higher levels being maintained during late summer and autumn (Australian seasons).

In Kenya, there is a marked seasonal variation in reproductive capacity in Merino ewes, as illustrated by the length of the oestrous cycle and the incidence of oestrus (author, unpublished results). These seasons are not sharply defined in different years, nor are they of the same extent, nor do they occur at exactly the same period in each year. Nevertheless this seasonal variation must, as far as possible, be taken into account in breeding operations. Some years and certain seasons of the year are more favourable for full expression of sexual activity in Merino ewes. In general, it may be said that it is in the early months of the year that the incidence of oestrus is highest and the cycle shortest in grade Merino ewes in Kenya.

It is therefore at this period of the year that the best results should be obtained from breeding operations. The results from artificial insemination in different seasons of the year support this view (Table 3). In a good year, however, the season favourable to reproduction may be greatly extended.

It is the normal practice in the Rift Valley district in Kenya to put the ewes to the ram in May and June. This season has been determined

solely by the welfare of the lambs, in that they do better and are less subject to helminth infection when born towards the end of the year. With better methods for control of worm infestation it may eventually prove advisable to alter the breeding season to a period somewhat earlier in the year.

One of the biggest difficulties in artificial insemination of sheep in Kenya has been the failure of ewes that did not conceive to return to the ram after the normal interval of about 17-19 days. If ewes which fail to hold to insemination (or service) do not return to the ram at normal intervals, they will have less chances for re-insemination during the breeding season and a poorer lambing will consequently result. In June-August 1936, only 25% of 159 ewes lambed, yet 75% of these ewes did not return to the ram. In the Experimental Station flock abortion is rare, but it cannot at present be decided whether or not conception and subsequent interruption of pregnancy may account for some of the cases of failure to return to the ram. The most probable explanation available at present, however, for failure of inseminated ewes to return to the ram, is the great seasonal variation that has been observed in the length of the oestrous cycle.

The percentage of conceptions to insemination during one heat period has never been greater than about 50%. In April to July 1939, for example, when each ewe was inseminated twice during the one heat period the average percentage of conceptions was 29%. To obtain the best results from artificial insemination in high-grade Merino ewes in Kenya, it is therefore essential to get as many ewes in lamb as possible when they first come on heat, and highly important, to ensure as far as possible, that those ewes which do not conceive will return to the ram after approximately the normal cyclical interval, for re-insemination. The main factor in determining this latter point is a seasonal one.

Even during a restricted breeding season changes occur in the reproductive activity of sheep. Grant's (1934) data show a distinct trend to longer cycles as the breeding season advances, and according to Chapman & Casida (1937), who examined these data statistically, this increase from month to month accounts for 37% of the variation in the oestrous cycle length in this group of sheep. McKenzie & Terrill's (1937) data also show that the length of the oestrous cycle increases during the breeding season. McKenzie & Terrill noted a general tendency for shorter oestrous periods to occur near the beginning and end of the breeding season. There is a tendency for shorter oestrous periods to occur near the beginning and end of the breeding season and the occurrence of ovulation

without oestrus has been noted preceding and following the breeding season (Grant, 1934; Cole & Miller, 1935; McKenzie & Terrill, 1937).

There is also evidence that the fertility of sheep changes during the breeding season. It has been observed that twins are usually born early in the breeding season (Heape, 1899; Marshall, 1905). In American flocks of Shropshire sheep, Roberts (1921) found that the percentage of multiple births was higher in the earlier part of the lambing season, and in British flocks, Nichols (1924) noted that by far the greater number of multiple births occur at a time corresponding to that at which the ewes go fastest to the ram, and although this time varies a certain amount according to the treatment the ewes and rams have received before mating, it is usually at the beginning of the breeding season. It thus appears, as Marshall states, that the reproductive activity of the ewe tends to be greatest at the beginning of the breeding season. It is not therefore surprising that, if variation in fertility occurs during a relatively short restricted breeding season, it should also occur in sheep, such as grade Merinos in Kenya, which can breed throughout the year.

Time of insemination. The optimum time for insemination depends mainly on (1) the vitality of spermatozoa in the genital tract of the ewe, and (2) on the vitality of the ovum. The consensus of opinion is that the life of the ovum is very short. Hartman (1932), in reviewing the evidence, states that 'facts are accumulating which show that the time of survival of the unfertilized ovum is measured in hours, not days.' The data of Quinlan *et al.* (1932) indicate that the ovum rapidly loses its vitality and is available for fertilization for a few hours only after ovulation. Kuznecov (1934) holds a similar view.

In grade Merino ewes in Kenya, ovulation occurs shortly after the end of oestrus, but there is a minimum period of about 23-25 hr. after the onset of oestrus before which it does not occur, even if the duration of oestrus is much less than this interval. Anderson (1938b) and McKenzie & Terrill (1937) found that, although there is some variation in the time of ovulation, generally speaking, it takes place near the end of oestrus. In Merino ewes Kelley (1937) found that ovulation took place after the end of oestrus.

The experiments reported in this paper on insemination after the end of oestrus, support the view that the life of the ovum is short. In the nine ewes whose mean oestrous period was 31.5 hr., ovulation presumably occurred about the end of oestrus. They were inseminated 1-13 hr. after the end of oestrus and after the probable time of ovulation, and none conceived. In the fifty ewes, whose mean oestrous period was

17.9 hr., ovulation probably occurred about 23–25 hr. after the onset of oestrus. They were inseminated 14–39 hr. after the end of oestrus and about 9–32 hr. after the probable time of ovulation, and none conceived. In grade Merino sheep sperm can retain its fertility for nearly 24 hr. and the findings of other workers indicate that sperm are capable of reaching the upper end of the Fallopian tubes fairly quickly. It therefore seems that the period for which the ovum of the ewe can be fertilized must be brief.

It is reported in this paper that spermatozoa from a Merino ram are capable of retaining their fertilizing capacity in the genital tract of the ewe for 22½ hr., the maximum period investigated in this experiment. Quinlan *et al.* have studied the vitality of spermatozoa of Merino rams in Merino ewes. In the vagina the majority of the spermatozoa are non-motile after 12 hr. Living spermatozoa were found in the cervix up to 48 hr. after coitus and it is believed that the cervix acts as a reservoir for spermatozoa awaiting the availability of the ovum.

These workers, assuming that the ovum is available for fertilization between the 36th and 40th hour (the interval after the onset of oestrus when ovulation is believed to occur), plus a period of a few hours taken by the spermatozoa to reach the Fallopian tube, state that spermatozoa are definitely capable of fertilization for 36–42 hr. after being deposited in the vagina. Green & Winters (1935), stated that spermatozoa do not live more than about 24 hr. in the genital tract. According to Polovceva *et al.* (1938), the average duration of survival of spermatozoa in the female genital tract may be estimated at 34–36 hr., but in several instances eggs were fertilized which had ovulated 40–50 hr. after insemination. Kelley (1937) found that the fertilizing power of spermatozoa from even the most fertile rams reached the threshold of infertility at approximately 34 hr. after copulation. The fertilizing power of the ejacula from the majority of the Merino rams had a shorter duration than that of the ejacula from the Dorset rams. In Kelley's (1937) experiment the limit of fertilizing power in hours post-coitus was 24 hr. for Merino rams. Further, there is evidence that the fertilizing power of the ejacula of Merino rams becomes reduced, if not lost, 10 hr. after coitus.

There is some difference of opinion on the time taken by ram spermatozoa to traverse the genital tract and reach the upper extremity of the Fallopian tubes. These times, following coitus, found by different workers are, (1) within 6 hr. (Quinlan *et al.* 1932), (2) about 5 hr. (Kelley & Dumaresq, 1936), (3) about 5 hr. (Green & Winters, 1935), (4) from 30 min. to 7 hr. 7 min. (Phillips & Andrews (1937), and (5) about 5 hr. in Merino ewes (Kelley, 1937).

Assuming that spermatozoa are capable of retaining their fertilizing capacity in the ewe for a certain period of time, it does not follow that they will be equally fertile throughout this period, although the few results given in this paper do not indicate any falling off in fertility after 22½ hr. It is probable, however, that fertility tends to diminish according to the length of stay in the genital tract, as is indicated by Kelley (1937). The vitality and fertilizing capacity of spermatozoa in the genital tract of the ewe are probably dependent on a number of factors which include the initial vitality and the capacity of spermatozoa for retaining this vitality and the condition of the genital tract of the ewe. It would appear from the work of Kelley that breed differences exist in the length of time for which spermatozoa retain their fertilizing capacity.

Number of inseminations. It is somewhat difficult to come to a definite conclusion about the number of inseminations required per heat period in grade Merinos. In actual practice the object is to ensure that there are available at the time of ovulation a sufficiency of spermatozoa of high vitality, and this, in view of the work already discussed could probably be attained by the introduction of spermatozoa at say, 5–6 hr. before the time of ovulation. Kelley (1937) comes to a similar conclusion. The effective life of spermatozoa in the ewe is, however, probably considerably longer than this, but it has not been accurately determined and the introduction of spermatozoa earlier than 5–6 hr. before the time of ovulation probably gives good results. Warbritton *et al.* (1937) stated that of three periods of insemination in the ewe, 12 hr. before ovulation was most desirable; for many ewes this meant breeding 10–18 hr. after the onset of oestrus. Kardymovic *et al.* (1934) found that the optimum time was 18–26 hr. after the onset of oestrus (the duration of oestrus not stated); at this time the percentage of conceptions was 84·8, and at 26–42 hr. after the beginning of oestrus the percentage of conceptions had fallen to 48, presumably due to a number of ewes having gone off heat before being inseminated. Zajac (1935) found that the optimum time was 24 hr. from the beginning of heat, but even at 48 hr. after the beginning of heat 77 % of ewes conceived. Since the time of ovulation is usually related to the end of oestrus, the optimum time for insemination depends on the duration of oestrus and this will vary in different breeds and under different conditions. Kelley (1937), for example, obtained a higher percentage of conceptions with matings within a maximum of 4 hr. from the onset of oestrus, in Merino ewes, than in Dorset ewes, which had a longer oestrous period.

When rams are run with the ewes, a ewe is probably served several times during one heat period. However, a single service has given similar

results to two or more services during the one heat period (unpublished results). Kelley (1937) found a difference in favour of several services. On the whole, in artificial insemination experiments at Naivasha, somewhat better results have been obtained by giving two or more inseminations in the one heat period, but the inseminations were not always carried out at the same season of the year and the results are not therefore strictly comparable. According to Milovanov (1934) the results of repeated inseminations have been excellent in some cases and negative in others. It is stated that it will be effective if the ewe is genetically capable of producing more than one ovum; if environmental conditions are favourable; if the interval between ovulations is long; and if the vitality of the spermatozoa is low. From experiments on 1100 ewes, Kirillov (1938) concluded that all ewes should be tested for heat twice a day, morning and evening and only ewes with a heat period of more than 24 hr. should be inseminated twice (the duration of heat in these ewes was under 24 hr. in 47 % of ewes, and 24-36 hr. in 45 % of ewes). It is stated that inseminating ewes with heat periods lasting over 1½-2 days, three or four times, does not raise the rate of lambing.

In grade Merino ewes at Naivasha, the mean duration of oestrus (1261 periods) was 26 hr. (author, unpublished results). In 1936, about one-third of the ewes began their heat periods between 6 and 9 a.m., another third between 9 a.m. and 3 p.m., and the remainder between 3 p.m. and 6 a.m. Since 1937 the usual practice has been to pick out ewes on heat, (1) in the morning and to inseminate them about 4 p.m. that day and again about 9 a.m. the following morning, and (2) during the day and inseminate them also at 4 p.m. that day and again about 9 a.m. the following morning. Thus, of ewes picked out in the morning about one-half have probably come on heat that morning and about one-half since the previous afternoon. For the former, the maximum duration of oestrus up to 4 p.m. that day would be 10 hr., and up to 9 a.m. the following morning 27 hr., and for the latter 25 and 42 hr. at these times. For ewes picked out during the day from 8 a.m. to 3 p.m., the maximum duration of oestrus at the times of insemination would be 8 and 25 hr. The upper limit for the interval since the onset of heat in the morning ewes is too high, for many ewes will have gone off heat by the time of the second insemination. For the day ewes, the upper limit is more satisfactory and it might therefore be expected that the day ewes would give better results. In the 1939 experiment, the differences were not great, the day ewes giving 32.4 % of conceptions and the morning ewes 29.2 %. In general, the procedure would seem to be appropriate for the majority of ewes, though the upper limit at the time of the second insemination may

be rather on the high side for some of the ewes. Since, however, as good results have been obtained with a single as with a double insemination in the one heat period, this question requires further investigation, and it may prove that in grade Merino ewes a single insemination at a favourable period of the year would be adequate.

Dilution. The extent to which sperm may be diluted depends mainly on the effect of the diluent on the spermatozoa and on the number of spermatozoa required for fertilization. Walton (1927) found that in the rabbit, fertility is influenced by the number of spermatozoa introduced into the vagina. The optimum dilution for various ram sperm diluents has been worked out. Diluent GPS-2, for example, which has been used in all the Naivasha experiments, has an optimum dilution of $\times 8$, but for practical purposes the addition of 1-3 parts diluent is advised (Milovanov, 1934). There is little information about the density of spermatozoa required for fertility in sheep. Milovanov *et al.* (1937) give the standard number of spermatozoa required for each insemination as 500×10^6 , but state that this varies from 1200×10^6 to 75×10^6 according to the resistance of the spermatozoa. (This number, however is for vaginal insemination; cervical insemination requires less.)

In pure-bred Merino rams in Kenya, the average number of spermatozoa per c.mm. is 2.5 millions and the average volume of the ejaculate is 0.72 ml., i.e. an average ejaculate contains about 1800 million spermatozoa. With the addition of 3 parts diluent to such an ejaculate, a dose of 0.2 ml. would contain 125 million spermatozoa, which might possibly be on the low side. In Naivasha experiments, there has not been on the whole, great differences between the use of diluted and undiluted sperm, but ordinary service has given a higher percentage of conceptions than the use of undiluted sperm. 70% conceptions were obtained from hand service compared with 47% from undiluted sperm in August 1936 (author, unpublished results). It is possible that this difference is due to the greater density of sperm introduced into the genital tract of the ewe in ordinary service rather than to better synchronization between the time of introduction of sperm and the time of ovulation, since both lots of ewes having been picked out in the morning were probably inseminated at approximately the same period of heat. Demidenko *et al.* (1933) also found a higher percentage of conceptions from ordinary mating, and little difference between sperm used diluted up to $\times 4$, and undiluted.

General. With ordinary service a higher percentage of conceptions is obtained per heat period than with artificial insemination. Therefore, provided a high percentage of ewes come on heat, it is easier to get a satisfactory lambing from ordinary service, even in less favourable

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seasons. With artificial insemination, when conditions are unfavourable to reproductive activity during the breeding season, ewes which fail to 'hold' take a long time to return to the ram. Their chances for re-insemination are consequently much reduced and a poorer lambing results. When conditions are favourable, the smaller percentage of conceptions at the first heat period from artificial insemination compared with ordinary service, does not prevent a good lambing, for the ewes return to the ram at the normal cyclical interval and can be re-inseminated twice or three times during the breeding season. It also seems, though the evidence is as yet somewhat inconclusive, that during a favourable season the incidence of conceptions per heat period from artificial insemination is higher than at other times of the year. The first essential for successful artificial insemination of high grade Merino sheep in Kenya is therefore to choose the best period of the year for the breeding season. At Naivasha this seems to be in the earlier part of the year.

The somewhat better results from two or more inseminations per heat period may be due to better synchronization between the times of introduction of sperm and the time of ovulation, but this is also as yet undecided, for single inseminations during certain seasons have given equally good and better results, and this may be due mainly to seasonal factors rather than to other reasons. Since a single service has given better results than a single insemination it would also seem that more attention should be paid to the number of spermatozoa introduced into the cervix at each insemination.

Ram. The fertility of rams varies considerably even when the character of the sperm is of the highest degree, according to present criteria. This constitutes one of the most important problems in artificial insemination. In the absence of a reliable criterion it is possible to obtain information on the actual fertility of rams by allowing them to serve a number of ewes before the breeding season and seeing how they 'settle' them. Uncompleted experiments indicate that there may be seasonal variations in reproductive capacity in the ram.

SUMMARY

The results obtained from artificial insemination of high grade Merino sheep in Kenya are given and discussed in relation to the season of the year, time of insemination during oestrus, the number of inseminations in each oestrous period and the degree of dilution of sperm.

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THE CAROTENE CONTENT OF CERTAIN SPECIES OF GRASSLAND HERBAGE

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(With One Text-figure)

THE importance of carotene in the food of animals requires no emphasis because it is one of the precursors of vitamin A. The most important source of vitamin A for livestock is green foodstuffs of which grass makes by far the outstanding contribution in the animal diet. Several investigators have determined the carotene in grass and whilst most of the work has been carried out on samples of mixed herbage there have been a few investigations on individual species (Atkeson *et al.* 1937; Synder & Moore, 1940; Moon, 1939*d*). All the evidence points to the fact that the carotene content of plants may vary considerably during the growing season and be influenced by manurial treatment. The object of the present investigation was to examine the effects of growth and manuring on the carotene contents of some of the most important species found in normal grassland; with that in view attention was given particularly to white clover (*Trifolium repens*), perennial rye-grass (*Ayrshire*) (*Lolium perenne*), cocksfoot (Danish) (*Dactylis glomerata*) and timothy (Scotch) (*Phleum pratense*). Samples were collected at different stages of growth from different farms during two seasons.

SAMPLING

Twelve of the fourteen farms from which samples were taken were in late districts and usually in exposed areas. The remaining two were in early districts. The mineral soils varied from light to heavy loams, the *pH* values from about 5-7.8, the available potash from very low values to moderately high values (as determined by the *Aspergillus* method), the available phosphate (by Kirsanov's acid extraction method) from a mere trace to values which would be considered moderately good. One farm was on deep peat which had recently been brought into cultivation and had received fairly liberal dressings of lime and artificial fertilizers. At

two centres the grass samples were obtained from experimental plots. In one case the experiment consisted of two series of plots on a soil originally well supplied with available potash but containing only a trace of available phosphate and with a *pH* value of 5. In one series a basal dressing of lime was applied, and different plots in replicate received different dressings of basic slag; in the other series, the plots received a basal dressing of slag and different plots in replicate received different dressings of lime. The lime and fertilizers were applied in July 1939, the grasses were sown shortly afterwards and grass samples were obtained in June 1940. In the other experiment, designed to test the effect of the late application of nitrogen on the protein content of hay, the soil was practically neutral in reaction and was moderately well supplied with both available potash and available phosphate. Nitrogen was applied to duplicate plots on 1 June 1940 at the rate of 1 cwt. per acre of ammonium sulphate and the grass was cut on 24 June.

In a few cases samples were obtainable from experimental plots of pure species, but otherwise the crop was mixed and the individual species were separated by hand. Sampling was started at the end of May. The plants were cut about 1 in. above ground-level with a knife, and the fresh material was transferred in waterproof bags as quickly as possible to the laboratory where the examination was carried out immediately.

METHOD OF ANALYSIS

Many investigations have been carried out on the extraction of pigments from plant material, and several writers have summarized the merits of the various methods proposed. Preference is now generally given to a separation of the chlorophylls from the carotenoids by saponification followed by extraction with petroleum ether and the disintegration of the tissue seems to be most easily accomplished by boiling with aqueous potash (Moon, 1939a). There is no doubt that the most accurate methods of determining the individual yellow pigments are spectroscopic and chromatographic. For rapid routine analyses, however, a colorimetric method is most desirable. At one time it was common to estimate the total carotenoids in solution and to calculate the carotene and xanthophyll from the ratio of these constituents in grass (Ferguson & Bishop, 1936). Preliminary studies showed, however, that this ratio is not constant; it depends for example, upon the temperature at which the green material is dried. Since, generally speaking, only the carotenes are provitamins, the determination of the xanthophylls, which can be completely removed

from the carotenes, may be omitted in the usual examination of green foodstuffs. The simplest method of separating the carotenoids is to make use of the fact that the carotenes are more soluble in petroleum ether than in methanol, while the reverse is the case with the xanthophylls. Methyl alcohol of 92% by volume is the most effective solvent to use. Quackenbush *et al.* (1938) have shown that several carotene-like pigments with no vitamin potency are produced by the action of acids on the carotenoids and it has been found by Seaber (1940) that 20-30% of the yellow pigment in the petroleum ether extract from dried grass is not carotene. According to Kon & Thompson (1940), however, the over-estimation of carotene by the colorimetric method, although as much as 30% for samples of hay, dried grass or silage, is only about 3% for fresh grass. The probability is that the carotene undergoes decomposition as a result of oxidation or heating or the action of acids. For these reasons it was decided to determine the carotene in samples of fresh material as soon as possible after collection.

After selecting the particular species required, a portion was dried in the oven for 24 hr. at 100° C. in order to determine the percentage dry matter and total nitrogen in the sample. All the figures have been compared on a dry matter basis on account of the unavoidable variation in the moisture content of samples, due to climatic conditions. Another sample was chopped into short pieces and thoroughly mixed and 5 g. were taken for the carotene estimation. This sample of 5 g. was boiled for 1½ hr. under a reflux condenser with 40 ml. of 20% aqueous potassium hydroxide. The mixture was filtered under reduced pressure and the residue was stirred up in a beaker with about 25 ml. ethyl alcohol and the liquid decanted through the funnel. This was done four times and followed by one extraction with 25 ml. of petroleum ether (b.p. 40-60° C.). The precipitate which forms by reaction between the different extracts in the filter flask was filtered off and washed with petroleum ether. The filtrate was shaken vigorously in a separating funnel and the lower aqueous layer separated from the ether layer. The aqueous liquid was then extracted three or four times with fresh portions of petroleum ether until the yellow colour in the last extract could be completely removed by 92% methyl alcohol; this showed that the whole of the carotene had been extracted from the aqueous layer. The collected ether extracts were then extracted with successive amounts of 20 ml. of 92% methyl alcohol until all the xanthophyll was removed. Finally the petroleum ether solution was washed twice with distilled water to get rid of any alkali present.

The total volume of the extract was measured and the colour of an aliquot was compared against Lovibond yellow colour standards in a tintometer. The curve prepared by Ferguson (1935), showing the relationship between β -carotene and Lovibond colour standards, was used for this purpose. From time to time the solutions were checked against a 0.1% bichromate solution in a Klett colorimeter using Ferguson's conversion factor. The results were calculated in terms of parts of carotene per million parts of dry material.

RESULTS

Part of plant. In many cases where the grass had reached flowering stage the heads were examined separately from the stems and leaves. The results in Table 1 show that, as might be expected, there was generally less carotene in the heads than in the leaves and stems. The reverse, however, is the case for all the rye-grass samples and for one sample of cocksfoot, possibly because of the relatively low values for carotene in the leaves and stems of these samples.

Table 1. Carotene (p.p.m.) in leaves and stems, and in heads or flowers

Clover		Cocksfoot		Rye-grass		Timothy	
L. and S.	H. or F.						
183	95	92	60	4	20	112	98
143	72	57	90	60	80	208	125
323	119	42	38	53	74		
		166	111	27	75		
		211	148	135	151		
				82	103		

Effect of manuring. The effects of manuring were not spectacular except in the case of the late application of ammonium sulphate to rye-grass hay. This was applied three weeks before cutting and increased the carotene content of the leaves and stems of the rye-grass from 64 to 99 p.p.m., and of the heads from 88 to 103 p.p.m. This result is in agreement with the results obtained by other workers and summarized by Moon (1939b).

The effects of slag and lime were not so marked. It may be noted that the soil on which this experiment was carried out contained only a trace of available phosphate and that it was impossible to obtain a sample of grass large enough for analysis from the unslagged plots. As is shown in Table 2, extra slag produced an increase in the carotene

content of the cocksfoot amounting to about 22% in the leaves and stems and about 8% in the heads. The corresponding increases for rye-grass were only 3 and 12% respectively. The addition of a large dressing of lime to the plots already receiving 10 cwt. of slag increased the amount of carotene in the leaves of the cocksfoot by about 11% but had little effect on the heads or flowers. In the case of the rye-grass, there was little effect on the carotene content of the leaves whilst that of the heads was increased by about 11%. As will be seen below, these effects were small in comparison with the effects due to differences in stage of growth.

Table 2. *Effect of phosphate and/or lime on carotene content (p.p.m.)*

(a) All plots received 5 cwt. per acre CaO.							
Cocksfoot				Rye-grass			
5 cwt. slag		20 cwt. slag		5 cwt. slag		20 cwt. slag	
L. and S.	H. or F.	L. and S.	H. or F.	L. and S.	H. or F.	L. and S.	H. or F.
111	90	135	97	74	85	76	95

(b) All plots received 10 cwt. per acre slag.							
Cocksfoot				Rye-grass			
2½ cwt. CaO		42½ cwt. CaO		2½ cwt. CaO		42½ cwt. CaO	
L. and S.	H. or F.	L. and S.	H. or F.	L. and S.	H. or F.	L. and S.	H. or F.
113	92	126	92	77	81	77	90

Site. No direct comparison was possible between the site and the composition of the plant because the date of sampling or the height of the plant could not be taken as criteria of the stage of growth in different localities. The results for any one site showed the enormous importance of stage of growth on the composition of the plant and when the results for each species were plotted against stage of growth for each locality the lines showed a marked parallelism. For these reasons it may be assumed that the part played by the soil is small in comparison with the part played by stage of growth. One comparison is sufficient in this connexion. One of the sites was on deep peat whilst the other sites were all on mineral soils. The average value for the carotene in all the clover samples was rather higher than that for the samples grown on peat whilst the reverse was the case for cocksfoot; the average values for both the rye-grass and timothy were practically the same in the two cases.

Species. An examination of the figures in Table 3 shows that white clover contains much more carotene than the other three species. Figures

for carotene range from 143 p.p.m. for a sample of old material taken in August to 552 p.p.m. for a sample of very young clover. In the case of cocksfoot, the figures vary from 42 p.p.m. for a sample which was fully ripe taken in July to 385 p.p.m. for a sample of grazed aftermath taken in September. The figures for rye-grass, with one exception, lie between 27 p.p.m. for a sample of old material taken in July and 261 p.p.m. for a sample taken in September after grazing. There is one value as low as 4 p.p.m. for some very old material taken in August; most of the seeds had been shed. In the case of timothy the values vary from 112 p.p.m. for a ripe sample taken in July to 275 p.p.m. for a sample of young aftermath which had been grazed by sheep.

Table 3. *Comparison of carotene (p.p.m.) and total nitrogen (% d.m.) in species at different stages of growth*

Stage of growth	White clover		Cocksfoot		Rye-grass		Timothy	
	N	Carotene	N	Carotene	N	Carotene	N	Carotene
Young	3.7	456	2.5	382	1.3	88	1.1	223
	3.8	509	—	—	—	—	—	—
	4.5	552	—	—	—	—	—	—
Flowering or heading	2.6	183	1.4	141	1.0	60	2.1	194
	3.0	323	2.0	211	1.0	60	1.6	208
	3.2	488	2.1	146	1.0	53	—	—
	—	—	2.3	166	1.7	82	—	—
Old	2.4	143	0.8	57	0.6	4	1.3	112
	2.6	252	0.9	42	0.7	27	—	—
	2.9	194	1.2	123	—	—	—	—
Aftermath	3.9	326	1.4	92	1.4	137	2.0	222
	3.9	304	1.8	138	1.2	114	1.2	171
	—	—	1.3	148	2.1	189	—	—
	—	—	1.0	102	1.5	138	—	—
	—	—	1.5	225	1.4	96	—	—
	—	—	1.7	300	—	—	—	—
Grazed	3.0	355	4.0	385	2.0	261	2.8	275
	3.9	454	2.3	258	4.3	254	3.1	247
	—	—	—	—	2.5	135	—	—
	—	—	—	—	3.1	225	—	—
Average	3.4	349	1.8	182	1.7	120	1.9	207

The word 'grazed' used in this table signifies that the grass sample was short because it was actually being grazed or had recently been grazed.

A comparison of the figures with the data obtained by Moon (1939d) shows that his values are about 50% higher. The reason probably lies in the fact that the samples under consideration here were not taken before May and sampling was continued until October the grass being cut only for hay at the normal season or grazed. Moon, on the other

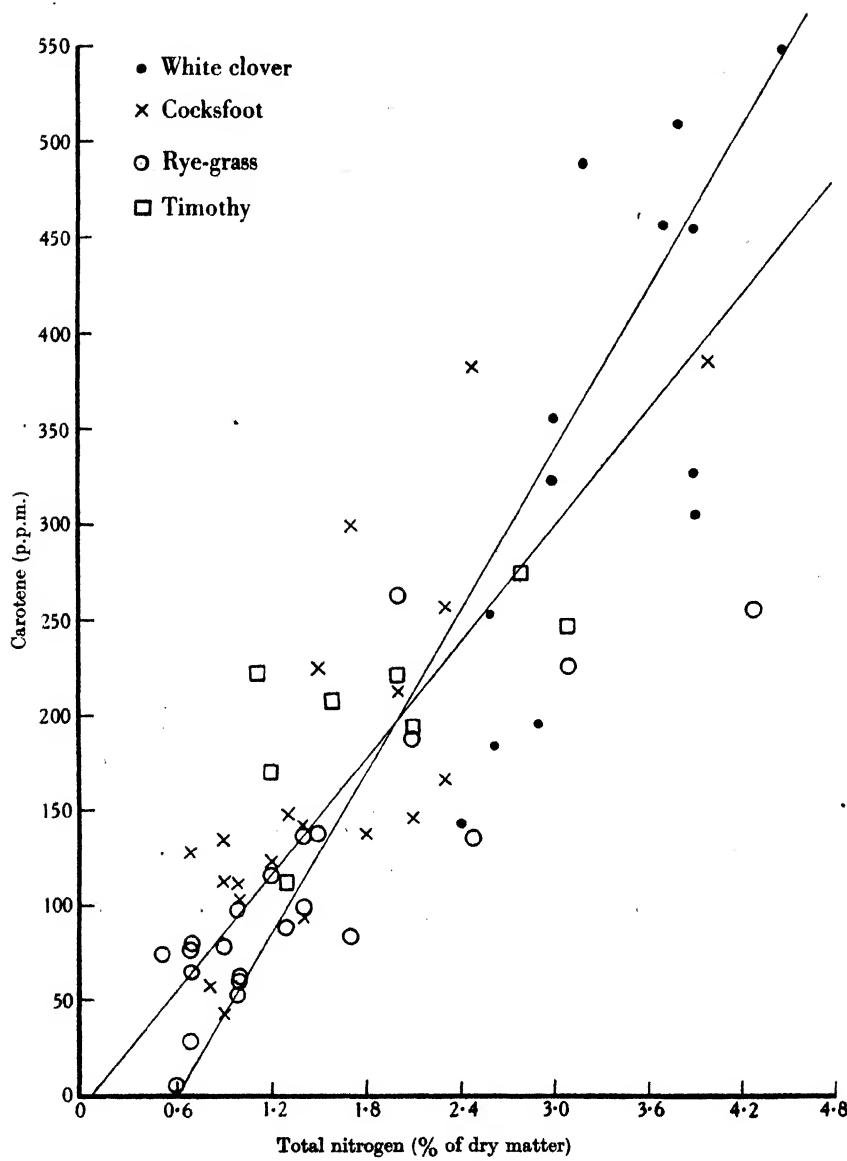


Fig. 1.

hand, started to sample at the end of March from nursery plots of pure species which were cut back twice during the season; furthermore, he took only the leaves for his samples in August. In spite of these fundamental differences in the growth and sampling of the species the order of the amounts of carotene is the same, decreasing from clover to timothy, cocksfoot and rye-grass, but his figures for rye-grass are relatively higher than those given here.

Stage of growth. With respect to the effect of stage of growth it will be observed that all four species show a similar variation. The carotene contents were greatest when the plants were young, tended to decline at flowering or heading, decreased rapidly when fading or ripening and increased again for the aftermath. This variation is not so marked with white clover and timothy as with the other two species where the results cover a wide range and were not quite so regular. For example, two of the earlier aftermath samples of cocksfoot contained rather less carotene than samples of older material taken from the same locality. This was almost certainly due to the fact that the young aftermath was not entirely free from dead grasses left after cutting.

Carotene and nitrogen. One of the most striking features of the results is the very close parallelism between the figures for total nitrogen and carotene. This association is obvious from the scatter diagram (Fig. 1), the actual correlation coefficient being +0.85 for sixty-three samples. This relationship has already been dealt with by Moon (1939c) who found a high degree of correlation between carotene and both true and crude protein for mixed herbage.

Grazing. Another very important observation which emerges from the work is the beneficial effect of grazing. This applies to all four species and to both nitrogen and carotene contents. It is unfortunate that samples could not be obtained in the very early part of the growing seasons but the results available show that both carotene and nitrogen were usually much higher in the grazed samples than in those at heading stage or in the aftermath samples. The effect is more striking in the case of the grasses than in the case of the clover, the nitrogen and carotene contents of the grazed samples being much higher than those for the samples taken in May and June.

SUMMARY

The total nitrogen and carotene have been determined in sixty-three samples of white clover, cocksfoot, rye-grass and timothy taken at different times during the growing season from various localities.

Except for rye-grass, there was more carotene in the leaves and stems than in the heads or flowers. A late dressing of ammonium sulphate increased the carotene content of rye-grass by 55 and 17% in the leaves and heads respectively. The effects of lime and slag on the carotene content of cocksfoot and rye-grass were much less marked and there were no obvious effects due to environment.

The predominant factor in determining both nitrogen and carotene, which were very closely related, was stage of growth. Grazing effected important increases in both constituents.

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BIOLOGICAL ASPECTS OF SOIL FERTILITY

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(With Plates 5-7)

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INTRODUCTION

UNDER the above title an expression of opinion concerning certain biological aspects of soil fertility was put forward by the writer in a recent number of *Nature* (Neilson-Jones, 1940). The present paper gives a detailed account of the experimental study of soils at Wareham Forest on which these opinions were based. The work owed its inception to researches by Rayner on the same area in which the observed infertility of these soils for tree growth is ascribed to causes quite other than their known deficiency in mineral nutrients, and in which there is offered a

biological interpretation both of their infertility and of the amelioration brought about by novel methods of compost treatment (Rayner, 1934, 1936, 1939). The difficulty of accounting for the observed growth phenomena along orthodox lines first challenged the attention of the writer, who is not a soil expert and makes no claim to such specialist knowledge; the point of view is that of a plant physiologist.

The area in question has been the subject of intensive research since 1932, and for the past three or four years has engaged the attention of a team of workers whose direction of attack has varied with their respective specialist interests. A great body of evidence has been thereby accumulated; part of this has formed the subject-matter of papers already published; other parts, such as that related to fungal growth and mycorrhizal response in conifers, will be published shortly. It is against this comprehensive background that the present paper, dealing with one only of the fundamental soil problems, has been written.

The region known as Wareham Forest is part of the Dorset Heath area now under afforestation by the Forestry Commission of Great Britain, and consists of poor *Calluna* heath on Bagshot Beds; it lies to the north-west of the town of Wareham between the valleys of the Piddle and the Frome, comprising some 3450 acres; it varies in elevation from 35 to 250 ft. above sea-level.

The geological history that has led to the formation of soils derived from estuarine Tertiary deposits such as these Bagshot Beds, with an account of the difficulties they present when attempts are made to utilize them in agriculture or forestry, has been admirably summarized by A. C. Forbes (Forbes, 1938). At Wareham, they consist usually of a few inches of heather peat over 12-24 in. of bleached sand, but the soil profiles show great variation, ranging from typical podsol to sand or silty clay resting directly on gravelly subsoil without pan (Pl. 5, figs. 1-3). Much of the surface peat is probably of recent origin, as there is evidence that a large part of the area was stripped of its covering within the last 100 years. The contours of the ground with the local occurrence of pan or fine subsoil affect the surface drainage, and flooding is still common after rain where drainage operations have not become effective.

In general physical characteristics the soil resembles those on similar Bagshot Beds elsewhere, as for example in the London Basin. It has, however, certain special characters very noticeable in handling for pot experiments. The colour is dark, black rather than the characteristic brown; it is 'greasy' in texture and stains the hands, the stains being difficult to remove; it is often malodorous, the unpleasant smell being

especially marked when the hard lumps that occur sporadically are freshly broken.

Not only is the growth of pines, sown or planted on the untreated soil, poor in general, extending to complete arrest, but the intensity with which these effects are exhibited varies widely and suddenly from place to place: isolated trees or groups of trees showing fair growth occur here and there on ground otherwise characterized by almost complete suppression of growth without any evident correlations with the more obvious soil features such as distribution of pan, etc. Nor can local inhibition of growth be interpreted as due to local deficiency of inorganic nutrients; no such correlation has been observed, nor would it explain why certain trees, the growth of which has remained in abeyance for some years, should suddenly and spontaneously start to grow. Moreover, in areas characterized by poor growth of pines, there is evidence of reduction in the number of species and activity of the fungi present; sporophores of the higher fungi are notably rare and mycorrhizal associations with pines deficient in number and abnormal in structure.

The behaviour of young pines at Wareham Forest and in pot experiments with transported soil, using untreated soil and that receiving alternatively an addition of mineral nutrients or one or other of the special composts referred to below, is confirmatory of the view that fertility of these soils is not mainly controlled by their mineral content, but rather by the character of the organic residues present (Rayner, 1939). In the untreated soil, these are—accepting Rayner's conclusions—to a varying extent toxic as reflected in the poor growth of the trees and the sparseness of the micro-flora both in species and numbers; on modifying these residues by appropriate control of the biological reactions producing them, for example, by compost treatment, the soil behaves as a fertile soil capable of supporting a vigorous growth of pines; at the same time there is evidence of biological regeneration, including marked activation of growth of the higher fungi, with resumption of normal mycorrhizal activity on the part of the young trees. These changes cannot be paralleled by directly making good the deficiencies in mineral salts. The alteration in character of the decompositions, once started, tends to be self-propagating. Hence, it is argued, the fertility of these soils appears to be governed by the nature of the organic residues derived from micro-biological action; fertility is here a biological rather than a chemical problem.

The views put forward by Rayner in the papers cited involve an hypothesis that the normal cycle of decompositions responsible for pro-

gressive breaking down and accumulation of the humus constituents is disturbed, thus leading to the formation of an organic substrate, aberrant in respect to the balance of its constituents, unfavourable to the growth of many micro-organisms, and therefore disturbing to the normal balance of the soil population—a feature directly manifested in the case of young coniferous trees by a more or less complete failure of mycorrhizal development. Implicit in this view is the appearance of substances, or 'toxins', actively inimical to plant growth. Assumption that the course of decomposition can be controlled by modification of the organic substrate led to the use of special composts as a 'means of biological control, setting free dormant activities and bringing about profound changes in the organic substrate' (Rayner, 1934).

This hypothesis of soil behaviour involves a new conception of the significance of *qualitative* differences of humus constitution as a potent factor directing the course of biological activities in the soil with consequential influence on plant growth, apart from effects associated directly with physical properties or with the presence of directly available mineral nutrients.

The plant physiologist is simplifying unduly the problem with which a plant growing in soil is faced in obtaining nutrients by means of its roots, if it be assumed that conditions approximate to those of experimental laboratory cultures in which mineral salts are supplied to the roots for absorption. In soil, the activities of various micro-organisms complicate the natural root environment in many ways: not only by direct competition, but by controlling the forms in which available nutrients are presented and the production of substances beneficial or deleterious to growth; also, by forming beneficent or harmful associations with the root tissues. The fact that probably 80% of flowering plants form mycorrhizal relationships is alone sufficient to suggest that the simple picture of mineral salts absorbed through root hairs represents only part of the mechanism of root nutrition—in many cases quite a small part of all the processes intervening between addition of organic matter to the soil and absorption of nutrient by the plant. Presumably, the difficulty of precise quantitative investigation of the nutritive relations of mycotrophic plants is responsible for retention of the convenient fiction that this form of nutrition is casual and exceptional, when in point of fact it appears to be normal and regular for a majority of mature flowering plants growing in soil.

SCOPE OF THE PRESENT ENQUIRY

In planning the researches, attention was directed especially to the following aspects:

- (1) Demonstration of the presence or otherwise of soil 'toxins' of biological origin actively inimical to plant growth.
- (2) If present, determination of their biological action in the soil, the manner of their formation, and the conditions favourable or otherwise to their production.
- (3) The mode of action of compost treatment in bringing about complete amelioration of the soil as a medium for tree growth.

Evidence bearing on these problems has been obtained from a number of different lines of approach.

Acceptance of the assumption as a working hypothesis that fertility as reflected in tree growth is related mainly with the character of the dominant micro-biological activities, led to the employment of new methods of biological analysis, consisting essentially in 'determination of the comparative reactions of the soil as a whole towards substances of known chemical composition or towards specific organisms' (Neilson-Jones, 1940). By use of these methods it is believed that information has been obtained as to the biological potentialities of different soils and as to the reactions taking place in them such as could not have been yielded by the standard methods of soil investigation or by study of soil organisms in pure culture.

Earlier observations had already pointed to the probable occurrence of a 'toxic' factor (Rayner, 1934). As judged from observations on plant growth in the field, the degree of 'toxicity' varies with extreme particularity from place to place and also at different seasons. It has been noted that the conditions associated with pot culture, e.g. leaching, improved aeration, etc., cause gradual amelioration of the unfavourable symptoms.

For purposes of comparison, the effects of Wareham soil on growth were measured against those of two other soils of like physical character and geological history, both with similar *pH* values. Of these, one from a woodland area in the New Forest is referred to in what follows as 'New Forest soil'; the other, from the Oxshott district in Surrey, as 'Oxshott soil'. These, unlike Wareham soil, support satisfactory growth of pines. The term 'toxic' is to be understood as signifying 'inhibitory to growth' without any implication that such action is necessarily lethal.

EVIDENCE OF SOIL TOXICITY: ITS REMOVAL, RESTORATION,
AND DISTRIBUTION

(1) *Volatile products from the soil*

In view of the amelioration that follows from leaching and improved aeration, an early attempt was made to ascertain if volatile toxic products could be extracted from Wareham soil. It was discovered that air passed over this soil heated on a water-bath induced epinastic curvatures of the petioles of tomato similar to those caused by traces of ethylene and certain other substances (Neilson-Jones, 1935). This effect was not produced by garden soil or by New Forest soil, neither of which are inhibitory to tree growth. Owing to practical difficulties of experimentation and the inconsistencies of the reaction, this line of investigation was abandoned when the more convenient mycological test method about to be described was discovered. The significance, if any, of the epinastic reaction in tomato in relation with those aspects of soil toxicity now under consideration has not yet been elucidated.

(2) *Toxicity to fungi: the nutrient-agar-film test method*

That there is an inhibiting effect on the growth of many fungi can be demonstrated very simply and clearly by the following method initiated by A. G. Morton.

An agar medium is made up to the formula of Melin's glucose agar: 2% glucose, 0.01% magnesium sulphate, 0.05% ammonium chloride, 0.1% potassium phosphate (KH_2PO_4), 2% agar-agar, with citric-phosphate buffer to bring the pH value to 4.7, an average value for Wareham soil. The soil sample to be tested is placed in a Petri dish and the liquid agar, just before setting, poured over the surface to form a thin film. If toxicity is present, the nutrient surface so formed remains free from mycelial growth for an indefinite period when exposed to casual air infection by removal of the cover. Freedom from mycelial growth is equally maintained after sowing with *Penicillium* spores or inoculation with mycelial transplants. No such inhibition occurs with nutrient agar alone, nor is it obvious in cultures with nutrient films overlying New Forest or Oxshott soils. It may be inferred, therefore, that some substance inhibitory to mycelial growth is present in Wareham soil and diffuses through the agar film. This behaviour explains the observed inactivity of fungal mycelium in the natural soil (Pl. 6, figs. 4, 5).

The acid character of the soil and agar medium is unfavourable to

bacterial growth in general; bacterial colonies sometimes appear in these cultures but are few in number; they are always relatively inconspicuous, and in the present account are ignored as irrelevant to the matters now under discussion.

The degree of toxicity of a soil sample can be estimated equally well by noting the growth of mycelium or germination of spores contained in soil inocula. If small particles of soil are plated on the surface of the nutrient agar, mycelium grows out from their edges *if the underlying soil is not toxic to growth; if toxic, no visible growth of mycelium appears.* When present in sufficiently high concentration, the toxin is lethal to active mycelium and completely inhibiting to the development of spores; there is no evidence that it is lethal to mycelium in a resting condition or to spores. It is immaterial, therefore, whether the inocula used are taken from a toxic or non-toxic sample of soil, the appearance or not of a visible growth of mycelium from the inoculum being determined only by the nature of the soil underlying the agar film.

The distinction between toxic and non-toxic soil is not sharply defined—there are varying degrees of toxicity. The character of the mycelial growth on the surface of an agar film, exposed to air infection or sown with spores, reflects such variations. When toxicity is absent or slight, a profuse growth is rapidly formed; with increased toxicity the surface of the film may appear to be free from mycelium to the naked eye, but examination with a lens reveals a sparse network of fine fungal threads. With still higher toxicity even these threads are absent and only ungerminated spores can be seen; that the spores are not killed can be demonstrated by removing portions of such a film from contact with the underlying soil and plating on the same agar medium, when spore germination can be observed. Furthermore, the inhibiting effect acts differentially as regards fungal species: e.g. the aerial mycelium of species of *Mucor* is more sensitive than that of species of *Penicillium*.

(3) *Instability of the toxin: impermanence apart from soil*

The inhibitory factor from underlying soil can diffuse through only a comparatively thin layer of agar. The photographs reproduced show cultures with agar films respectively 3 and 6 mm. thick 7 days after exposure to air infection, maintained under precisely similar conditions; the former shows very slight, the latter copious, mycelial development (Pl. 6, figs. 6, 7). There is evidence from other experiments that the inhibitory substance is rapidly removed, either volatilized or oxidized or otherwise chemically transformed, so that unless constantly renewed its

effects quickly disappear. In the experiments just described, disappearance of the inhibitor evidently occurs from one of these causes in passing through the agar, with the result that the concentration at the surface of a 6 mm. layer of agar is insufficient to overcome the vigour of fungal growth induced by the nutrients present. Because of this instability it has been found necessary to collect and transport soil samples for laboratory examination with as little disturbance as possible, and to maintain them carefully under controlled conditions.

It is a point of considerable interest that field sampling shows that toxicity is associated not only with the surface humus layer, but also with the underlying leached layer to a depth of 30 cm.; it is absent from the lower dark layer at a depth of 35-40 cm.¹

(4) *Removal and restoration of toxicity: its biological origin*

The inhibitory effect on growth is removed immediately from soil by treatment of various kinds: autoclaving at 120° C. for about 20 min., steaming for a minimum period of 20 min. (but not exposure to a temperature of 50° C. for 1 hr.), treatment with alcohol, ether, toluol, or similar antiseptics either by irrigation or by exposure to vapour for several days. Tested by the nutrient-agar-film method, cultures of soil subjected to any of these treatments soon develop a copious growth of mycelium following exposure to air infection. Not only so, but cultures similarly treated without an agar film soon carry *directly on the soil surface* a vigorous growth of fungal colonies after exposure to casual air infection or deliberate inoculation. In soil cultures without an overlying agar film, this development of a superficial growth of mycelium is doubtless favoured, not only by elimination of the toxic factors, but also by reduced competition and by liberation of food materials by the methods of sterilization or partial sterilization employed. In the corresponding cultures with an agar film, however, the superficial mycelium is separated from the soil by a layer of agar containing excess nutrients, so that neither extra supplies of food material in the soil nor competition for these by soil organisms can be of importance, but the behaviour of the superficial mycelium must depend mainly on the extent to which toxic substances are produced in the underlying soil and diffuse through the agar film. That diffusion of inhibitory substances rather than starvation or competition is the main factor responsible receives confirmation from the results of experiments in which toxicity of the soil is reinduced after it had been removed by one or other of the above-mentioned methods.

¹ A. G. Morton, unpublished observation.

Air-drying has an effect on soil somewhat similar to steaming: on remoistening, the toxic reaction is found to have disappeared and mycelium grows as readily though with less vigour than on steamed soil. In fact, complete air-drying may be regarded as a mild form of partial sterilization, the capacity of the soil for supporting a vigorous superficial fungal growth subsequently being due, as with steamed soil, to removal of the toxic reaction from the soil and the provision of an additional source of food—in this case presumably from the organisms killed by desiccation.

Whether borne on the soil directly or on overlying nutrient films, the mycelial growth on cultures treated by any of these methods persists for many weeks if kept moist.¹ It is suggested that the fungal growth on sterilized soil results solely from increased food supply whatever its origin, it must be pointed out that the agar film contains excess sugar and inorganic nutrients yet no such growth takes place when overlying *untreated* soil, from which it may be inferred that provision of nutrients is not in itself sufficient to bring about the observed result, but that removal of toxins is also a potent factor. Final and conclusive proof that the inhibiting effect of the soil on growth is brought about by the presence of by-products of microbial activity, effective even in the presence of additional food liberated during sterilization or provided in an agar film, is given by the behaviour of sterilized soil subjected to inoculation compared with that of the same soil not inoculated. A culture of partially sterilized soil, inoculated with a fragment of untreated soil and exposed to infection, with or without an overlying film of nutrient agar, develops a copious fungal growth on the soil or nutrient agar as before. But in this case, the aerial mycelium begins to fail at about the 6th day and by the 10th day from inoculation has completely disappeared (Pl. 7, figs. 8-13). In those cultures in which early mycelial growth is carried on a superficial nutrient agar film, the subsequent mycelial retrogression can hardly be due to competition for available food materials by organisms introduced with the inoculum, but must be ascribed to diffusion through the agar film of a substance derived from the inoculated soil. It is a legitimate inference that the rapid failure of growth and collapse of mycelium simultaneously over the whole surface of the culture that occur also in absence of an agar film are due to the same causes.

It is justifiable to infer that the toxicity so induced is of biological origin; that with the soil inoculum has been introduced an organism that spreads throughout the soil, producing as a by-product of metabolism

¹ If kept too wet toxicity may return (see p. 389).

some substance that reaches a concentration sufficiently high in about a week to inhibit visible fungal growth; and that this organism is killed or inactivated by the 'sterilization' treatments employed and accumulated toxic products volatilized, oxidized, or otherwise removed.

This cycle of behaviour can be repeated, i.e. 'sterilized' soil, having regained its toxicity as a result of inoculation with untreated soil, can be 'resterilized' with loss of toxicity, and the toxicity can be again induced by reinoculation. The vigour of fungal growth on soil that has had its reaction reversed several times becomes reduced, however, doubtless from exhaustion of food materials; repetition of the cycle appears to be limited only by the amount of food material available.

(5) Irregular distribution of toxicity in soil

Restoration of toxicity by inoculation with untreated soil has been observed in a large number of experiments, each involving many cultures. It must therefore be accepted as a phenomenon of general occurrence. Occasionally, however, a soil culture receiving an inoculum in the form of a small fragment from an unbroken lump failed to show any return of toxicity. As an extreme example may be quoted an experiment consisting of twenty cultures of which three failed to redevelop toxicity after inoculation.

Such behaviour appeared to indicate that the organism responsible is not distributed uniformly throughout the soil, so that if small inocula are used, these may be occasionally without it. This interpretation is supported by the fact that toxicity never fails to reappear if large inocula are used or if the soil sample is thoroughly mixed before inocula are taken.

The particulate nature of soil toxicity as exhibited by growth of pines in the field and the inferred irregularity of distribution of the organism concerned in soil samples, add considerably to the laboriousness of experiment, since it becomes imperative to replicate every experiment many times in order to ensure reliable conclusions. The final results now recorded are based on a large number of cultures receiving similar treatment: many of the disconcerting inconsistencies and contradictions met with in earlier stages of the investigation are now regarded as due to failure to appreciate that a sufficient number of cultures must be used in every experiment to eliminate chance effects due to irregular distribution in soil samples.

ORIGIN AND CONTROL OF TOXICITY

One of the fundamental difficulties encountered at an early stage of this investigation was the provision of comparable samples of toxic soil. As already stated, an area from which samples showed a high degree of toxicity might provide little evidence of this when tested a month later. Research was therefore directed to determine the conditions that favour so conspicuously the activity of the toxin-producing organism in Wareham soil at certain seasons. Field observations had already indicated a definite seasonal periodicity; assuming normal seasonal temperatures and precipitation, the deleterious effect appears to reach a maximum in mid-January, decreasing from March throughout the summer with an upward tendency from October onwards. Bearing in mind that toxicity, as judged both from the behaviour of young trees in the field and from that of fungi in laboratory cultures, is at its maximum in late winter and decreases during the summer, the effects of low temperature and of high water content, with its accompanying poor aeration, were investigated.

(1) *Low temperature and development of toxicity*

By the kindness of the Cambridge Low Temperature Research Laboratory, a large number of samples were kept in closed bottles at about 4° C., one or more of the samples being withdrawn at intervals for comparative estimation with that of corresponding samples kept at laboratory temperature. Since no evidence was forthcoming that low temperature alone affected the reaction of the soil, it is unnecessary to describe these experiments in further detail.

(2) *Moisture content of the soil and development of toxicity*

In order to determine the effect of varying moisture content, experiments were set up with untreated soil (toxic) and the same soil steamed once (non-toxic) under conditions ranging from comparative dryness to wetness sufficient to produce waterlogging. The results of many such experiments may be summarized as follows.

Dry or relatively dry samples of untreated soil gradually lose the capacity to check fungal growth, after a time behaving in this respect like once-steamed soil. With increased moisture content toxicity persists, samples kept for a month or longer in a waterlogged condition developing toxicity to an even greater degree than is observable in 'bad' samples from the field.

It may be concluded, therefore, that high water content with its

accompanying poor aeration provides a condition extremely favourable for the development of toxicity. This is in accord with the inference that the organism concerned is anaerobic in habit, and also with field observations on seasonal fluctuation in degree of toxicity.

It may be objected that no proof has been offered that toxicity induced in laboratory cultures by waterlogging is identical in origin and nature with that observed in the field and in soil samples transported to the laboratory with due precautions. This is admittedly the case: since, however, maximum toxicity in the field coincides with or closely follows those seasons of the year when maximum waterlogging occurs and minimum toxicity with those seasons that provide the reverse conditions, and since corresponding variations of toxicity are developed by laboratory cultures kept under varying conditions in respect to moisture content, it is a reasonable inference that the two sets of phenomena are due to the same cause. This does not preclude the possibility that deleterious substances are produced by causes other than waterlogging, or that basic causes other than those affected directly by bad aeration may play a part in determining the degree of toxicity exhibited. The immediate point of interest is that toxins are produced under waterlogged conditions in the field and under similar conditions artificially imposed in the laboratory, that these cause similar inhibition of fungal growth, thereby bringing about grave disturbance of the microbiological equilibrium within the soil. Treatments that remove these inhibiting factors in the field or in pot culture lead to rapid establishment of soil fertility as measured by the growth reactions of young trees and other vascular plants. There is at present no direct evidence that the toxins shown to be responsible for inhibition of fungal growth act directly on the growth of vascular plants.

(3) Partial and complete sterilization in relation to toxicity

The discovery of a method for controlling production of toxicity in the laboratory has supplied an essential factor for rapid and continuous experimental progress, inasmuch as it immediately overcomes the difficulties and delays occasioned by the variation in degree of toxicity of soil collected in the field at different seasons. It ensures that samples of fully toxic soil shall be available whenever required, even at times when such are difficult or impossible to collect in the field.

Since untreated soil kept in a waterlogged condition maintains or even increases its toxicity, the effect of high water content on soil treated in various ways was investigated. The experiments led to the following conclusions:

(i) Soil that has been air-dried or steamed once for not more than 20 min. is rendered non-toxic and remains so for an indefinite period if kept no more than moderately moist; under conditions of extreme moisture it gradually regains toxicity.

(ii) Soil that has been steamed for an hour or longer, autoclaved, or soaked for several hours in alcohol fails to regain toxicity spontaneously under waterlogged conditions—it requires inoculation with untreated soil before doing so.

Such behaviour points to a resistant stage in the life history of the organism responsible for toxicity.

(4) *Types of organisms associated with toxic soil*

No attempt has been made to survey completely the microflora of Wareham soil. The following facts are available in regard to the experimental area.

Of the higher fungi, sporophores of *Boletus bovinus*, *Laccaria laccata* and *Thelephora terrestris* occur; those of *Boletus* are abundant locally in the neighbourhood of young pines that have started spontaneously into vigorous growth and also near those growing in composted plots.

As judged by microscopic examination, mycelium is relatively scarce in the soil; that of members of the hymenomycetes can be identified, also that of *Mycelium radicum atrovirens*, often associated with abnormal mycorrhizal development in pine (Melin, 1923). Somewhat unexpectedly, mycelium of *M. r. nigrostrigatum*, recorded by Hatch (Hatch, 1934; Rayner, 1935) as most frequent in less fertile soils, particularly in those with low or slowly decomposing organic content, has never been observed on the experimental area as a mycorrhiza-former except where introduced with humus inocula.

Soil plated on various culture media of pH range between 4.8 and 8.0 (including beerwort agar, peptone agar and yeast agar) has yielded regularly *Thamnidium* sp., *Mucor racemosus*, *M. hiemalis*, and several species of *Penicillium*; it is doubtful if the latter are true soil species.

The inhibitory effect of Wareham soil on growth has been tested on *Boletus bovinus*, *Mycelium radicum atrovirens*, *Rhizoctonia silvestris*, members of *Mucoraceae* and *Penicillium* spp. Of these, *Rhizoctonia* appears to be absent from Wareham soil except where introduced, but it is an important member of the microflora at Hope Forest in Yorkshire, an area showing many of the phenomena of growth inhibition characteristic of Wareham Forest; there it is responsible for a form of root infection which is undoubtedly deleterious to tree growth.

The Mucoraceae are highly susceptible, *Penicillium* spp. much less so; consequently, development of even moderate toxicity in the soil results in the disappearance of all mycelial growth due to members of Mucoraceae, while that due to *Penicillium* spp. disappears only when the soil has become highly toxic. As regards the three fungi that can form root associations, *Boletus bovinus* is very susceptible, *Mycelium radicis atrovirens* and *Rhizoctonia* sp. are comparatively resistant; this no doubt accounts for the pines growing in Wareham soil showing abnormalities of mycorrhizal structure instead of forming normal healthy mycorrhizas with *Boletus bovinus*.

With regard to the bacterial flora, soil plated on the various media used has produced very few bacterial colonies. We are indebted to Dr A. Thaysen for the following observations on two samples of Wareham soil submitted to him for bacterial examination. The total number of micro-organisms growing on agar media is definitely low, a noticeable feature being the preponderance of aerobic soil bacilli which are present in a ratio of four to one of the total types of organisms. Both these features may be regarded as an indication of 'lack of life' in the soil. No evidence was found that *Azotobacter* is present. A marked feature is the presence of H_2S -producing bacteria, evidence of which was obtained in samples even so small as 0.1 g. 'This is almost equal to the numbers to be expected in clay soils where H_2S -formation is particularly noticeable.'

The occurrence of sulphur-reducing bacteria in Wareham soil in unexpectedly large numbers led to the following experiments.

(1) A series of cultures of once steamed soil was set up in flasks or Petri dishes of which half received an addition of 0.1% NaCl solution, the remainder a corresponding addition of Na_2SO_4 solution, each culture receiving sufficient liquid to produce a waterlogged condition. A period of 12 days was allowed to elapse during which stimulation of the activity of the H_2S -producing bacteria might take place, after which all cultures were exposed to air infection. A month later, all flasks that had received NaCl solution still showed a profuse growth of *Mucor* spp. and *Penicillium* spp., whilst those that had received Na_2SO_4 solution showed either no mycelial growth or a very sparse growth of *Penicillium* spp., any fungal development that had appeared in the earlier stages having retrogressed.

The increased toxicity of soil to which sulphate is added is explicable if the toxicity is related in any way with a slow and continuous evolution of traces of H_2S derived from the activity of bacteria capable of reducing sulphates. This interpretation is purely speculative in so far as it is based on an isolated observation.

(2) Dishes containing toxic soil and a superficial nutrient agar film were prepared in the way previously described. In half of the cultures strips of lead acetate paper were embedded in the agar before it had solidified; in the other half of the cultures strips of plain filter paper were embedded similarly. All cultures were exposed to air infection. At the end of a week, the control cultures with filter paper were entirely free from mycelial growth; the other cultures were also free from mycelium except above the strips of lead acetate paper where fungal colonies had developed. The absence of toxicity in this region was not due to the strip of paper acting as a mechanical barrier to toxic substances diffusing from the soil as shown by the behaviour of the control cultures; it suggests rather that the lead acetate, diffusing into the surrounding agar, removes H_2S by chemical reaction before it can reach the surface of the film. This interpretation may seem at variance with the fact that the strip of lead acetate paper did not blacken, but two ways may be suggested of meeting this difficulty. In the first place the traces of H_2S sufficient to inhibit fungal growth may be too small to produce a visible precipitate of lead sulphide; and in the second place the lead acetate with which the test paper is impregnated will diffuse into the agar, so that any lead sulphide formed by H_2S diffusing from below will be deposited in the agar under the paper in close contact with the dark-coloured soil where its presence cannot be recognized. The possibility, however unlikely, that lead acetate might stimulate fungal growth was tested in further cultures; these showed that no stimulation occurred, but suggested that differential suppression of growth might take place since when lead acetate was used the flora was mainly *Penicillium* spp. with little or no *Mucor* spp.

(3) A number of other observations are in conformity with the view that H_2S is concerned in the production of toxicity. For example, it has been observed that the mycelium of air-borne fungi is highly sensitive to H_2S , the smallest trace of this gas being sufficient to check growth. Again, in the early stages of work on the use of nutrient films over soil, it was noted that gelatine behaved quite differently from agar, mycelial growth occurring readily on the surface of a gelatine film in contact with a sample of soil that completely suppresses growth on a corresponding film of nutrient agar. If the inhibitory substance is H_2S this differential behaviour receives explanation, since gelatine and H_2S show considerable capacity for combination.

Although these observations are in conformity with the view that the inhibiting substance is H_2S , and it is known that organisms capable of

producing H_2S in suitable culture media occur plentifully in the soil, the presence of this gas in Wareham soil has not yet been proved by direct chemical methods. Work is being directed towards this end and towards isolation of a sporing member of the sulphur-reducing group of soil bacteria. That H_2S -producing organisms are responsible for the inhibitory action of Wareham soil is put forward at the present stage as a suggestive speculation and basis for further research.

The interpretation at present attached to the experimental results bearing on the origin and nature of a factor directly inhibiting fungal growth in Wareham soil may be summarized as follows:

- (1) The toxin is a by-product of the metabolism of some soil organism.
- (2) The activity of the organism or organisms concerned is greatly favoured by waterlogged conditions; this is evidenced both by direct observations of behaviour in laboratory cultures and by inference from observations in the field where the inhibiting factor is known to occur to a depth of 30 cm. Occurrence in the field is sporadic; the conditions responsible for this have not been fully determined.
- (3) The organism is inactivated but not killed by drying, by steaming for short periods and by other methods of partial sterilization of soil; it can be reactivated by keeping the soil under waterlogged conditions.
- (4) The organism is killed by autoclaving for half an hour at 120° C., by long and repeated steaming, and by prolonged exposure to antiseptics. The toxicity of a soil sample treated by any of these methods can be restored only by inoculation with untreated soil.
- (5) It is reasonable to conclude that a sporing bacterium anaerobic in habit may be the biological factor concerned.
- (6) In view of the occurrence of sulphur-reducing bacteria in numbers unusually large for poor organic heath soils of this type, experiments were devised to determine how far the toxic effects exhibited by this soil could be attributed to the continuous production of small traces of H_2S . These show that (1) small traces of H_2S are extremely potent in inhibiting mycelial growth in many fungi; (2) treatment of the soil in ways that might be expected to favour the production of H_2S by micro-organisms leads to increased toxicity; (3) treatments that would have the effect of removing any H_2S present also remove the inhibitory effect of the soil sample on fungal growth.

(5) Distribution of the toxin-producing factor in different soils

The presence of a toxin-producing organism in Wareham soil raises the question of its wider distribution. The following experiments were

designed to supply an answer to this question. Samples of Wareham soil and Oxshott soil were subjected to partial sterilization by steaming for 20 min. Three sets of cultures of each soil treated as follows were then set up.

- (W. 1) Sterilized Wareham soil.
- (W. 2) Sterilized Wareham soil inoculated with untreated Wareham soil.
- (W. 3) Sterilized Wareham soil inoculated with untreated Oxshott soil.
- (O. 1) Sterilized Oxshott soil.
- (O. 2) Sterilized Oxshott soil inoculated with untreated Oxshott soil.
- (O. 3) Sterilized Oxshott soil inoculated with untreated Wareham soil.

All cultures were exposed to air infection and kept under observation for mycelial growth. The results were as follows:

- (W. 1) and (O. 1) developed profuse and persistent mycelial growth.
- (W. 2) developed mycelial growth which began to die within a week and in 10 days had disappeared.
- (W. 3) developed mycelial growth which persisted for about 10 days and then slowly died off.
- (O. 3) behaved like (W. 3), but the mycelium died off more slowly.
- (O. 2): the mycelial growth persisted for a longer period than did that on (W. 2), but otherwise behaved similarly to (W. 2).

The results of this set of experiments, many times repeated, using cultures with soil alone or with overlying nutrient films, indicate that the organism responsible for the development of toxicity is present in Oxshott soil but exists therein in a less active condition. For this reason an inoculum of Oxshott soil takes longer to induce the toxic condition than one of Wareham soil. The unexpected result that sterilized Oxshott soil slowly develops toxicity when inoculated with the same soil untreated suggests that sterilization favours growth of the toxin-producing organism as it does that of air-borne fungi, the slower development of toxicity in this case compared with that when Wareham soil is inoculated with Oxshott soil being due to the less favourable substrate provided by Oxshott soil. It is realized that these results are complicated by the fact that with the soil inoculum will be introduced many organisms as well as those credited with the production of inhibitory substances, and that any interpretation must take into account that competition from these organisms may have a depressant effect on the growth of the superficial mycelium apart from inhibition due to toxins. But these results have been obtained in cultures in which the superficial mycelium was carried on an overlying nutrient agar film, in which case the nutrients supplied in the agar should

counteract depression of growth from starvation due to competition within the soil. The factors operating in experiments of this kind are obviously of extreme complexity and there must be uncertainty as to their correct interpretation until the results of further analyses with many kinds of soil are available. The present enquiry is concerned mainly with the toxic phenomena associated with Wareham soil: the experiments just described are of interest in this connexion inasmuch as they are in complete conformity with the hypothesis that the difference between Wareham soil and Oxshott soil in this respect is one of degree only, the conditions in Wareham soil being specially favourable to the toxin-producing organism.

In experiments of this kind it is obviously impossible to attach numerical values to degrees of toxicity. The observed rate of growth or disappearance of aerial mycelium in any culture is a resultant of soil factors favouring growth on the one hand and depressing it on the other. In practice, it is impossible to control all such factors; consequently the rate of change in mycelial development on a toxic soil is strictly comparable only with that of a control set up at the same time on the same soil sample. A similar experiment set up at some other time will show the same order of events but the time scale may be different. For example, as pointed out on p. 389, increased moisture content of the soil markedly accelerates the development of toxicity; therefore two cultures of sterilized soil taken at different times from the same soil sample and differing as to moisture content would both show a return of toxicity subsequent to inoculation, but differ in the period required for such toxicity to be manifested. Possible variation in the mycelial flora derived from air infection introduces another variable, although this has not been found to affect the fundamental results; in order to establish this conclusion, experiments with cultures subjected to air infection were checked by corresponding cultures sown with spores of the same fungal species growing in pure culture.

CELLULOSE DECOMPOSITION

Arrest of the micro-biological activities responsible for cellulose decomposition is definitely indicated by Rayner as part of the hypothesis put forward to account for soil infertility; this assumed 'accumulation of a peaty layer having a relatively high proportion of cellulosic constituents as compared with proteins and lignins' (Rayner, 1934). Suggestive as is the theoretical argument in support of this view, no direct

evidence was offered that such accumulation of cellulosic residues actually occurs. The provision of experimental proof that Wareham soil contains a substance depressing or completely inhibiting to fungal growth invited enquiry as to whether this depressant effect extends to the organisms responsible for cellulose decomposition, since it was already known that the bulk of cellulose breakdown in Wareham soil to which compost had been added was due to fungal activity (see p. 400).

The following record of a preliminary experiment carried out by A. G. Morton, in which pads of cotton wool were buried in pots of soil and examined at the end of four months, shows that decomposition of cellulose is abnormally slow in Wareham soil. The condition of the pads as determined by visual inspection, resistance to tearing, discolouration, etc., is given in Table 1.

Table 1

Treatment	Condition of cotton-wool pad after four months
1. Wareham soil—untreated	No apparent alteration
2. New Forest soil—untreated	Considerable reduction in bulk, discolouration and loss of fibre strength as judged by resistance to tearing
3. Wareham soil + 25 % compost C 5	Complete decomposition; no recognizable remains of cotton-wool pad to be found
4. Wareham soil + N.P.K. salts in the proportion present in C 5	Somewhat similar to that recovered from New Forest soil, but rather less action: reduction in bulk and loss of fibre strength with some discolouration

Experiments carried out under field conditions gave similar comparative results, although the actual rate of decomposition was considerably slower owing to less favourable conditions in respect to moisture and temperature as compared to those constantly operating in laboratory and greenhouse culture.

For the above experiments, cellulose was used in the form of cotton-wool pads each weighing approximately 7 g.; these tended to obstruct drainage thus leading to drying out of the soil below. For this reason in later experiments filter paper absorption blocks (Whatman no. 11 grade, 14 x 16 mm.) were used in place of the cotton-wool pads. In both cases the cellulose was buried 1-2 in. below the surface of the soil, and the cultures kept moist and at laboratory temperature.

The absorption blocks were weighed after drying at 80° C. and were found to be fairly uniform in weight, each being about 1.1 g. At the close of the experiment the blocks were removed, washed carefully in a slow stream of water, and dried at 80° C. After drying, a little loose soil can

be sometimes shaken out of the block. The weight of the block after this treatment may be regarded as approximating closely to that of the cellulose residues; error from failure to remove completely adherent soil is small and will tend to be greatest in blocks suffering most breakdown and so cause the amount of decomposition that has actually occurred to be underestimated rather than exaggerated.

Many sets of such experiments have been carried out, the corresponding series all giving like comparative results. The results given in Table 2 may be regarded as representative; they are from a series that included soils from two sources. The experiment was started early in March and brought to an end 24 weeks later.

Table 2

Treatment	Weight of individual blocks at end of experiment g.	Mean weight per block g.	Percentage cellulose decomposed in 24 weeks
Fresh blocks dried at 80° C. (not used in experiment)	0.99 1.09 1.25 1.08	1.102	—
In Wareham soil—untreated	0.95 0.91 1.00 1.10	0.990 (statistically insignificant)	<10.0
In Wareham soil + 25 % compost C 5	0.10 0.18 0.08 0.03	0.098	91.1
In Wareham soil + 25 % leached C 5	0.75 0.65 0.77 0.65	0.705	35.9
In Oxshott soil—untreated	0.75 0.80 0.70 0.67	0.73	33.6

In addition to the above, cultures were used with Wareham soil steamed, autoclaved and dried, and with similar cultures inoculated with untreated Wareham soil and Oxshott soil; in none of these was any appreciable amount of cellulose decomposed.

From these results it may be concluded that the activity of the cellulose-destroying organisms is almost entirely inhibited in Wareham soil; considerable cellulose destruction occurs in soils of similar character such as those from Oxshott or the New Forest. Addition of compost causes an enormous acceleration in the rate of cellulose decomposition;

part of this may be ascribed to the water-soluble fraction containing inorganic nutrients and any growth substances produced by fungal activity; but the effectiveness of the leached compost shows that there is a 'compost effect' apart from this. How the compost may operate to bring about increased cellulose decomposition together with amelioration of the soil is discussed in the next section.

EFFECTS OF COMPOST TREATMENT

(1) *Effect on fungus growth*

The rapid improvement in Wareham soil brought about by addition of organic composts is believed to be complex in origin, depending on the interaction of factors acting directly and indirectly on growth. It was inferred by Rayner that one of these factors is the disappearance of a substance directly inhibiting root growth of the young trees with removal of the biological causes responsible for its production. No direct independent proof was offered of the existence of such deleterious substance; the provision of evidence associating it in any way with the growth-inhibiting toxin now demonstrated would supply welcome correlation of observations made in the course of two independent researches. A first step towards obtaining evidence as to this is to test by the mycological methods now available the effect on fungal growth produced by addition of compost to the soil: for this purpose the compost made from hop-waste known as C 5 was selected as the most suitable.

When tested by the nutrient-agar-film method, the behaviour of toxic soil to which C 5 has been added in the proportion of 25% by volume resembles that of the same soil rendered non-toxic by partial sterilization; in both cases the overlying film carries a strong mycelial growth a few days after exposure to air infection or direct inoculation. In the absence of the nutrient film, however, cultures of soil mixed with compost, in marked contrast to those of partially sterilized soil, show little *immediate* capacity for supporting fungus growth directly on the soil surface. It is perhaps not surprising that the compost, while removing the factor or factors inhibitory to growth, does not supply the nutritive requirements for immediate mycelial activity, since the materials of the compost heap have been subjected to vigorous fungal action and the resulting product used when such action has slowed down from depletion of the supply of readily available nutrients. The vigour of fungal action at the earlier stages of the composting process is evident from the abundance of mycelium present and the profusion of sporo-

phores intermittently produced on the surface of the heaps. A relatively early stage of decomposition is marked by great activity of thermophilic organisms, indicated by the development of temperatures ranging from 50 to 70° C. It is believed that the chemical changes brought about during this stage of composting are of critical importance in determining the value of the final product. A later stage is marked by a prevalence of hymenomycete mycelium and sporophores. These observations are in agreement with those made by Waksman and his colleagues (Waksman *et al.* 1939).

We are indebted to Dr F. A. Baker for extending our own observations on the character of the cellulose breakdown in respect to the organisms causing it. Employing his special methods of examination in polarized light, he reports that the bulk of the cellulose decomposition both in compost heaps and in Wareham soil to which compost has been added is due to *fungal* activity (Baker & Martin, 1937).

(2) *Effect on the soil*

The effect of compost in removing inhibition of mycelial growth from any soil sample must be distinguished from that responsible for bringing about permanent amelioration in respect to the growth of vascular plants. Removal of toxic symptoms might result from physical effects such as adsorption or improved aeration, or might be due to chemical neutralization, or to a change in the balance of biological activities. Since addition of charcoal to the soil fails to reduce toxicity, the first possibility seems unlikely, although no doubt some changes in aeration may result from admixture with compost. The experiments with lead acetate and gelatine indicate that inhibiting substances can be removed by chemical neutralization (p. 393); it is possible that some of the organic constituents of the compost react in this way. But permanent amelioration of the soil such as actually takes place requires not only removal of toxic substances present, but also the bringing to an end of their further production. To accomplish this a change in the biological activities of the soil is necessary. It is suggested as an hypothesis that the organic constituents of the compost induce such change, promoting differential stimulation of the micro-flora, those elements of it ultimately responsible for the production of toxic substances being less favoured. The fact that compost after sterilization gives equally effective results¹ proves that it operates by modification of the activities of members of the micro-flora already present rather than by the introduction of new elements. If soil

¹ A. G. Morton, unpublished.

reaches a condition in which toxic substances are produced in amounts sufficient to inhibit general micro-biological activity, it may be surmised that such conditions tend to be self-propagating. Addition of compost breaks this vicious circle allowing establishment of an active humus decomposition, the products of which do not differentially favour the activity of the toxin-producing factor.

That the fundamental effect of the compost is to alter profoundly the soil bionomics is proved by the enormously accelerated rate of cellulose breakdown and the stimulation of the fungus flora in general in soil that has been treated with compost. That the basis for these changes lies in the organic constituents of the compost rather than in the inorganic nutrients it contains is evidenced by the efficacy of leached compost, by the differential effects of composts differing only in respect to organic constitution, by the dissimilar effects wrought by the same composts on different organic soils, and by direct experiment with additions of salts of nitrogen, potash and phosphoric acid equivalent to those contained in a given compost (Rayner, 1939). It is suggestive that the growth-promoting action of compost on fertile garden soils is often negligible or comparatively small; here presumably conditions are already favourable to fungal activity so that additions of compost can do little to improve fertility by modification of the micro-flora.

(3) *Effect on growth of pine seedlings*

The dramatic increase in the growth of various species of pine that results from additions of compost and the persistence of this effect, both in the field and in pot cultures, has been described and discussed in the papers already cited (Rayner, 1934, 1936, 1939). Since establishment of a mycorrhizal association is an invariable characteristic of healthy growth in pine, the question at once arises as to whether the beneficent action of compost on nutrition is directly on the tree or indirectly by way of the mycorrhizal association; the possibility of indirect action through stimulation of soil fungi in general must also be borne in mind. The problem of the mode of action of compost on growth of the trees is thus of considerable complexity, since any or all of these factors may be concerned.

There is abundant experimental evidence that the growth of pine trees is accelerated by introduction of an appropriate mycorrhiza-former into a soil from which it was previously absent or in which the mycelium was in a condition unfavourable to the establishment of a balanced mycorrhizal relationship (Rayner, 1934; Hatch, 1937). Although mycelium of *Boletus bovinus*, a known mycorrhiza-former of Scots pine, is present,

short root formation and therefore mycorrhizal development is defective in the root systems of pines growing in Wareham soil. It has been proved by direct culture that *Boletus bovinus*, like other soil fungi, suffers inhibition of growth in Wareham soil, removal of the inhibiting substances producing at once a favourable growth reaction. Addition of compost is one of the several ways in which toxicity to fungal mycelium can be removed from the soil; such addition to soil in which pines are growing is followed by great stimulation of root growth and the rapid production of mycorrhizas *proved to be due to association with B. bovinus*,¹ there can be little doubt, therefore, that one of the causes of benefit to the young pines is due to the action of the compost in promoting this result.

One of the immediate results of applying compost to Wareham soil is an enormous increase in the number of short roots produced by pine seedlings growing in the treated soil; the production of these short roots must greatly increase the absorptive efficiency of the root system. Apart from specially imposed experimental conditions, all these short roots are mycorrhizas (Rayner, 1936, Plate V). Since mineral salt solutions are known to have a depressant effect on the production of lateral roots (Mitchell, 1934), the increased development of these organs must be associated with some other constituent of the compost, acting directly on the seedlings or indirectly by stimulation of fungal activity in the soil.

The inference to be drawn from the marked increase in short root production that follows inoculation with humus containing active mycorrhizas or with mycelium from pure cultures is that the mycorrhizal fungi in the course of their active growth in the soil produce substances stimulatory to the growth of lateral roots (Rayner, 1934, 1941; Hatch, 1937). It is likely, therefore, that the increased short root production observed is due to the greater activity of mycorrhizal and other fungi in composted soil; possibly also to stimulatory substances already in the compost when applied. Intense fungal activity accompanies the composting process, and it is now well known that many fungi in the course of their metabolism give rise to growth-controlling substances. There is no evidence at present that the observed stimulus to short root production is to be ascribed to causes other than those resulting from vigorous fungal metabolism whether during the composting process or engendered in the soil by compost treatments. The nature of any growth-controlling substances present requires further analysis and is still under investigation. Although the results of compost applications to Wareham

¹ M. C. Rayner, 1941.

soil may be dramatically apparent in the growth reactions of pines, it is believed that these reactions are secondary in the broad sense, and that the fundamental effect is on the micro-biological relationships.¹

On the evidence at present available, general conclusions as to the origins and nature of the marked and permanent improvement wrought in Wareham soil by the addition of compost may be summarized under four headings as follows:

(1) *Removal of toxicity.* The importance of this in relation to renewed fungal activity and the part played by such activity in promoting soil fertility have been clearly indicated in earlier pages.

(2) *Addition of mineral nutrients.* Some of the composts used, notably C 5 now under discussion, contain relatively large amounts of available sources of nitrogen, potash, and phosphoric acid. Pot experiments of seedling pines to which mineral nutrients were supplied alternatively with composts in the proportions in which they occur in two different composts of which that now under discussion was one, prove that the major part of the improved growth cannot be ascribed to this cause; in some respects, as in suppression of short root formation, the effect of mineral nutrients may be deleterious. Permanent amelioration of the soil is not induced by this means. In field experiments at Wareham, addition of phosphoric acid in certain forms, e.g. bonemeal and basic slag, induces greatly improved growth although the immediate effects on growth are not comparable with those of composts either in magnitude or regularity. Whatever the degree of permanent amelioration, a matter now under investigation, there is evidence that, as with composts, an important part of the action of phosphatic fertilizers on Wareham soil is indirect by stimulation of certain elements of the soil population (Rayner, 1939; Howard, 1940; Morton, unpublished work).

(3) *Addition of growth-promoting substances of fungal origin.* The stimulus to root production following compost treatment, as well as that resulting from humus or fungus inoculation of soil, is regarded as ultimately of this origin. The compost itself probably contains such substances derived from the intense fungal activity accompanying its preparation, and induces their formation by stimulating the activity of soil fungi including those responsible for mycorrhizal association. Evidence respecting the existence and nature of growth-promoting substances in composts is not yet complete.

¹ An experimental study and analysis of the compost effect on seedling pines has been carried out by A. G. Morton and will be recorded in a separate publication.

(4) *Alteration in the constitution of the organic substrate.* This involves profound modification of the soil bionomics, is self-propagating, and brings about fundamental changes in the soil economy with corresponding results on the capacity of the soil as a source of nutrients for higher plants. Such change in the capacity of the soil to support plant growth is reflected directly, not only in the immensely increased growth of young pines, but also in that of grasses and other species that appear as weeds on the composted plots. While such changes probably affect mycotrophic plants in a special way, there is evidence that they extend also to non-mycotrophic species and are therefore of a quite fundamental kind in respect to soil conditions affecting plant growth and therefore to fertility in the broadest sense.

DISCUSSION

The researches recorded here originated in an attempt to determine the causes of the failure and inconsistencies of tree growth at Wareham Forest, Dorset. Among the causes of infertility suggested in earlier researches was the formation during humus decomposition of substances directly inimical to growth. The identification of such toxic substances appeared to be a necessary first step, since no positive evidence was forthcoming as to their actual existence, and because, if present, the nature and extent of their action must determine the direction and scope of further enquiry.

The outcome, as the foregoing pages have shown, confirms the hypothesis that toxic substances of biological origin occur in the soil, and proves that they operate directly by inhibiting fungal growth. It is believed that this inhibition of fungal growth reacts secondarily on the trees, restricting root growth, impeding mycorrhiza formation to the advantage of possible inimical root associations, and curtailing their supply of nutritive requirements normally rendered available as a result of fungal activity. But the effect of suppression of fungal activity as observed in Wareham soil is more fundamental and far-reaching than its immediate reaction on tree growth; enduring effects are brought about in the soil by accumulation of undecomposed humus residues and by diversion of normal degradation changes into channels leading to the formation of by-products deleterious or useless so far as vascular plants are concerned—it leads, in short, to the formation of infertile soil.

It was not altogether unexpected that attempts to approach the problem by purely chemical methods served mainly to emphasize the elusive character of the incidence and distribution of the inhibiting

factors. A first requisite for successful laboratory investigation was to find a method for evaluating the degree of toxicity of any soil sample. This has been provided by the nutrient-agar-film method, a most important and valuable means of investigation in that it recognizes that soil is a dynamic system, and provides an index of changes taking place within it without interference with biological activities. No doubt the method will be found capable of extension and elaboration.

The use of a technique based on it has shown that the toxic effects of Wareham soil are associated with micro-biological activity; that these effects disappear on destruction or inactivation of the biological factor concerned, and return on its reintroduction by inoculation or reactivation by suitable conditions of culture. The differential results following partial and complete sterilization of the soil indicate that the organisms responsible for the toxic effect have a resistant stage; the conditions that favour rapid development of toxicity point to an anaerobic habit.¹ The use of this technique has shown further that it occurs, not only in soils such as those at Wareham Forest and Hope Forest that contain amounts of inhibitory substance sufficient to affect fertility to a marked degree, but also in similar soils elsewhere, such as those at Oxshott, that present little indication of growth inhibition. By subjecting the latter to suitable conditions they can be made to develop a considerable degree of toxicity (p. 395).

The facts brought to light in respect to Wareham soil are believed to be of wide application: they do not apply only to similar infertile soils elsewhere; they can be related in a general way with degenerative changes taking place in woodland and other organic soils not showing marked symptoms of toxicity, but already in the earlier phases of changes leading to infertility from the causes indicated and in danger of becoming fully toxic ultimately unless the direction of the predominant micro-biological activities is modified. Their relevance to the problems presented by cultivated soils needs investigation. The importance of the issues in relation with crop requirements is sufficiently evident (Howard, 1940).

Much attention has been bestowed on methods for controlling the course of decomposition changes and the nature of their resulting products in manure and other organic detritus (Niklewski, 1935), and more recently, on various aspects of the procedure to be followed in the preparation of suitable forms of compost from many different kinds of organic waste (Howard & Wad, 1931; Howard, 1940). The remarkable

¹ Localized scarcity of oxygen in the soil may result, of course, from micro-biological action as well as from purely physical causes.

contrasts offered by extreme types of humus breakdown in temperate climates was stressed by Falck (Falck, 1923), but, in general, less attention has been given by soil specialists to the corresponding micro-biological activities taking place *in situ* in soil (Waksman, 1931).

The present investigation emphasizes the importance of differential biological activities in a natural soil as affecting its fertility. Qualitative differences in the resultant products may be determined by the action of varying local conditions on these activities at any stage; such differences direct the course of subsequent humus breakdown and, as at Wareham, the final effect may be far-reaching on soil fertility. In the natural soils that have come under observation, there is no doubt that fertility, as measured by the growth of coniferous trees or by the vigour of fungal activity, depends on maintenance of correct biological balance brought about otherwise than by increased supply of mineral nutrients (Rayner, 1939). In the laboratory, this biological balance can be modified by selective elimination of different groups of micro-organisms by various treatments; in the field, the most potent means of guiding biological activities is control of the litter or organic detritus that reaches the soil and determines the character of the nutrient available for the soil micro-organisms. This is influenced in the first instance by the vegetational covering and, in forestry operations, can be modified by appropriate planting or selective elimination; where the degenerative changes in soil fertility have progressed beyond a certain point, it may be easier and quicker to initiate a change by application of suitable organic materials. In the case of the soils under consideration, the results attained by this means are far more universally applicable and more permanent than the occasional benefits that follow addition of mineral nutrients. The former treatment removes toxicity and encourages the growth of those fungi concerned with the breakdown of humus material and of the hymenomycete species responsible for the production of normal mycorrhizas in pines and other trees. Direct evidence for this stimulation of biological activity is provided by the vigorous breakdown of cellulosic material, and by such evident signs of change in the treated soil as alteration of colour from black to brown, emergence of fungus sporophores, profuse formation of mycorrhizas, with concomitant signs of reactivation such as appearance of earthworms; in short, by removal of the biological inertia that is so marked a feature of the untreated soil. Confirmation is thus provided for Rayner's original diagnosis of the causes of infertility in this soil, and for the point of view that dictated the use of organic composts as a means of rapid amelioration.

It is evident that field treatments such as promote improved drainage and aeration *in course of time* will contribute to soil amelioration by reducing those anaerobic activities responsible for the production of toxic residues.

The identity of the organisms causing inhibition of growth in Wareham soil is still uncertain. There is evidence that sulphur-reducing bacteria may play a part, the effective substance in this case being H_2S . There are a large number of soil bacteria capable of filling this role and of liberating H_2S from organic matter or sulphates. It is evident that the anaerobic conditions that favour the production of this gas in this manner favour also its undue accumulation by impeding the activity of such members of the sulphur-oxidizing group of soil bacteria as may be present. Moreover, sulphur-reducing bacteria, if active agents, may not be the only organisms concerned in the formation of down-grade products of humus decomposition that contribute to the observed inhibition of growth. Complete elucidation of this aspect of the problem calls for independent and highly specialized research.

Study of the causes of soil infertility at Wareham Forest justifies the generalization that in organic soils of the acid type, possibly in all soils, maintenance of the activity of fungi, whether those responsible for the breakdown of organic detritus or those specialized forms concerned in the production of mycorrhizas, is of critical importance. It is not suggested that organisms belonging to other groups are not vitally concerned, but it appears that fungi form an essential link in a mechanism whereby organic detritus is incorporated into the humus of a fertile soil, and that therefore their activity can be used as an index of fertility; infertility may result either from absence of appropriate fungi or the presence of factors hindering their activity.

Chief among initial causes of inhibition of mycelial activity in natural soils is probably low oxygen pressure which depresses fungal activity directly and may do so indirectly from the nature of the by-products of anaerobic metabolism. Deleterious by-products in the soil may be slow to disappear even when the causes responsible for their accumulation, such as lack of oxygen, are no longer operating.

Absorption by roots, being a process dependent on the action of living cells, is directly affected adversely by absence of oxygen. A survey of the area at Wareham Forest made it clear that the inhibition of growth observed there was not due solely to such direct action. The work now recorded shows that factors of many kinds are concerned, and provides evidence that plant growth may be affected adversely or the reverse

through causes inherent in the soil bionomics, members of the soil population showing great sensitiveness to aeration and to the nature of additions to the substrate, whether derived from their own metabolic activities or from external sources.

The prescription of compost treatment advocated by Rayner to correct a special case of infertility in a natural soil clearly envisaged removal of unfavourable by-products with alteration of the soil processes responsible for producing them, in addition to promotion of mycorrhiza formation. The remarkable and persistent improvement in tree growth that followed from this treatment brings the experimental results at Wareham into line with records recently provided describing the action on crop growth of composts manufactured by the Indore process when applied to cultivated soils in many parts of the world (Howard, 1940). The great emphasis laid by Howard on the significance of mycorrhizal activity is a welcome and long-overdue recognition from the practical side of the important part played by this habit in crop nutrition; the indifference displayed to this aspect of soil research by soil specialists is difficult to understand. But the mycorrhizal habit, although widespread, is not universal in vascular plants; the soil possesses fertility in its own right as reflected in the growth of non-mycorrhizal as well as mycorrhizal plants. It is the free action of fungi that is regarded as essential for the development of soil fertility; that some of the species concerned also form mycorrhizal associations with certain of the higher plants may be to the advantage of the latter, but this is additional to and different from the generalized service that fungi, mycorrhizal or not, perform in the soil—it provides an alternative channel uniting mycelial activity in the soil with the nutritive processes in vascular plants.

The analysis of a particular case recorded in the present researches shows very clearly some of the ways in which a condition of biological inertia in the soil, however produced, may bring about infertility for growth of the higher plants, and has yielded direct evidence of some of the ways by which additions of organic material operate in restoring fertility.

It is realized that there are gaps in the argument as presented in this paper; it could hardly be otherwise in view of the complexities involved. Nevertheless, the evidence is so striking that disturbance of microbiological equilibrium in the soil by inhibition of fungal activity is a potent factor in bringing about infertility for growth of vascular plants, its direct relation with the results of forestry researches so impressive and the possibilities of wider application so evident, that publication at the present stage of the work appeared to be justified.

SUMMARY

1. These researches were undertaken in order to analyse and define with precision the causes of infertility in a heath soil at Wareham Forest, Dorset.
2. The presence of substances actively inimical to growth has been confirmed and the origin of the resulting toxicity established.
3. A new technique for biological analysis, the nutrient-agar-film method, makes it possible to estimate the relative degree of toxicity in any given sample, thus facilitating development of methods of laboratory control for elimination and redistribution of the factor or factors responsible.
4. It has been proved that the toxic substances are of biological origin and that they operate directly by inhibiting fungal growth. The resulting biological inertia is exemplified by almost complete cessation of cellulose decomposition. Following upon this inertia are indirect effects of a secondary character on growth of the higher plants, in the case of trees restricting root growth, impeding mycorrhiza formation, and curtailing supply of nutritive requirements.
5. Justification for the use of organic composts for relieving the observed infertility under field conditions has been provided by laboratory experiments proving that removal of toxicity and profound alteration of the organic substrate and soil bionomics follow addition of compost. Changes so induced are self-propagating and the effects on growth persistent.
6. The bearing of these results on fertility in other natural soils and in cultivated soils is discussed.

Acknowledgements are due to the Agricultural Research Council for the provision of a grant extending over three years which permitted the full-time co-operation of Dr A. G. Morton and the mycological collaboration of Dr I. Levisohn. To Dr Morton is due the initiation of the nutrient-agar-film method and the cellulose decomposition experiments. The results referred to in the present paper in so far as they are derived from detailed exploitation of the former owe much to the mycological skill and tireless work of Dr Levisohn. Other aspects of the investigation which include a large part of Dr Morton's researches will be dealt with separately.

Valuable help has been received from Dr A. C. Thaysen in the form of bacteriological examination of soil samples, and from Dr F. A. Baker

in the application of special optical methods in the determination of the nature of the decomposition changes undergone by cell-wall residues in Wareham soil and in composts; also from the Macaulay Institute for Soil Research for undertaking a formal analysis of a Wareham soil profile¹.

Finally, whilst the writer is responsible for the views here expressed, these views owe much to discussion with colleagues, above all with Dr M. C. Rayner whose co-operation at all stages of the investigation and whose outstanding pioneer work at Wareham Forest has provided data without which the researches described in the present paper could not have been undertaken.

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¹ As far as exchangeable cations are concerned, only in the surface layer is there any Ca or Mg worth speaking of—elsewhere the values are infinitesimal. The phosphate values are extremely low, so low that available P_2O_5 would only be recorded as a trace. This extract from the Report sufficiently indicates the poverty of Wareham soil in available mineral nutrients. The full Report is filed at the Macaulay Institute, Craigiebuckler, Aberdeen.



Fig. 1

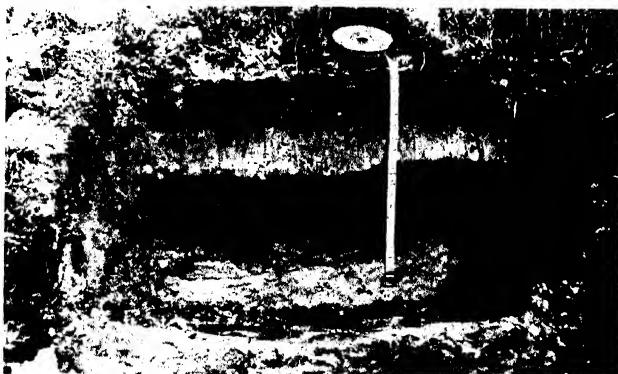


Fig. 2



Fig. 3

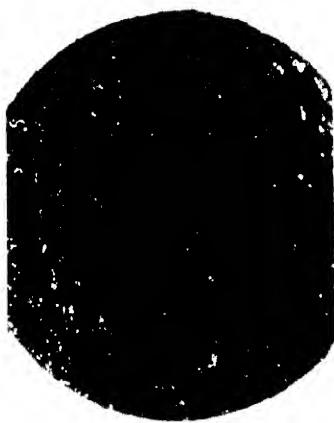


Fig. 4



Fig. 5



Fig. 6

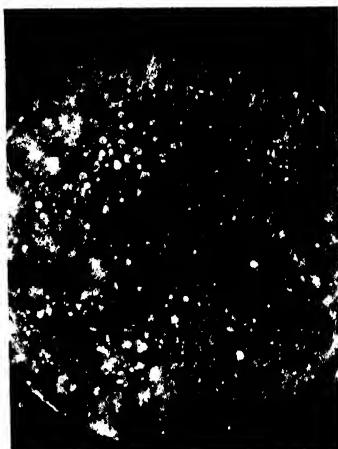


Fig. 7



Fig. 8

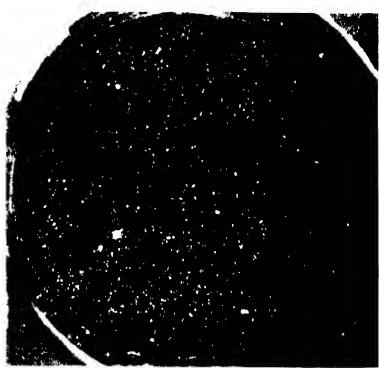


Fig. 9



Fig. 10

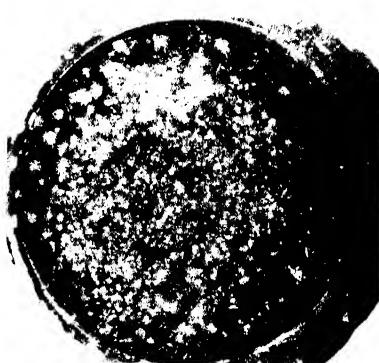


Fig. 11

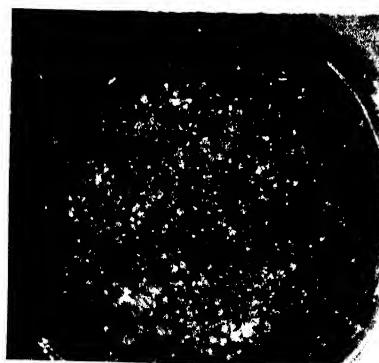


Fig. 12

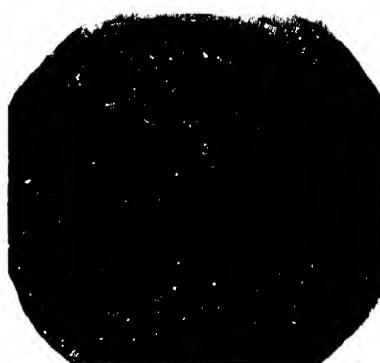


Fig. 13

EXPLANATION OF PLATES 5-7

PLATE 5

Figs. 1-3. Soil profiles from pits on different parts of the experimental area, Wareham Forest, showing variation in A and B horizons; in all three locations the soil exhibited toxicity. Fig. 1, thin *Calluna* peat resting directly on silty clay; Fig. 2, typical podsol; Fig. 3, *Calluna* peat resting on hard, light-coloured sand traversed by irregular dark bands.

PLATE 6

Figs. 4, 5. Comparative fungal growth on nutrient agar films overlying 'toxic' Wareham soil and Oxshott soil respectively; 10 days after exposure of the plates to air infection. (Inspection of the original of Fig. 4, Wareham soil, with a lens shows that the light specks are due to reflexions from soil particles; there is no mycelium visible.)

Figs. 6, 7. Comparative mycelial growth on layers of nutrient agar respectively 3 and 6 mm. thick overlying Wareham soil; 10 days after exposure of the plates to air infection. The inhibiting substance from the soil can cause considerable inhibition of growth at the surface of a 3 mm. layer of agar (Fig. 6), but is unable to penetrate 6 mm. of agar (Fig. 7). Compare Fig. 4 showing complete inhibition on a thin film of nutrient agar overlying the same soil.

PLATE 7

Figs. 8-13. Representative cultures from series receiving three different treatments.

Figs. 8, 9. *Wareham soil, untreated*. The same plate 12 and 28 days respectively after exposure to air infection; no development of fungal growth. (Absence of mycelium is due to the toxic reaction of the soil and not merely to paucity of available nutrients, since supplying these in the form of a nutrient agar film does not lead to development of mycelium; see Fig. 4.)

Figs. 10, 11. *Wareham soil, alcohol sterilized*. Fig. 10, profuse mycelial growth 12 days after exposure of the plate to air infection; Fig. 11, the same plate after 28 days showing continuation of vigorous growth. (This treatment removes the toxic reaction from the soil.)

Figs. 12, 13. *Wareham soil, alcohol sterilized and subsequently inoculated with the same soil untreated*. Profuse mycelial growth follows exposure of the plate to air infection. Fig. 12, 12 days after infection; initial growth still vigorous. Fig. 13, the same plate 28 days after infection; mycelial growth completely suppressed. (Suppression of growth due to increasing toxicity following introduction of the responsible biological factor in the small inoculum of untreated soil.)

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THE CARBONIC ANHYDRASE ACTIVITY OF THE HEN'S OVIDUCT

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IN their original paper on carbonic anhydrase Meldrum & Roughton (1933) drew attention to the possible significance of carbonic anhydrase in shell secretion in birds. As a first step towards testing this hypothesis a series of carbonic anhydrase estimations has been carried out on certain tissues of hens, using the simple open manometric method of Meldrum & Roughton (1933).

The birds were anaesthetized with 0.75-1.2 grains nembutal given intravenously, bled from the external jugular vein and then perfused with warm gum saline through a cannula inserted into the right ventricle. Aqueous extracts of the tissues still gave a faint benzidine reaction after this treatment, although extracts made with phosphate buffer, pH 6.8, did not.

Tissue extracts were prepared by grinding 0.5-0.8 g. tissue with finely crushed glass and 2 ml. water. For the manometric estimations of carbonic anhydrase activity 0.1 ml. of the extract was ordinarily used, although in some cases the original extract had to be diluted.

Blood extracts were made by taking 2 ml. oxalated blood in 50 ml. water, adding 10 ml. of 9% NaCl and making up to 100 ml. 0.1-0.2 ml. of this solution were used for the estimation.

All estimations were checked from time to time by HCN inhibition.

The results are set out in Table 1.

The values for blood are of the same order as those reported by Meldrum & Roughton (1933), for other warm-blooded vertebrates.

The values for tissues other than blood are low when compared with the data of Meldrum & Roughton (1933) and of Davenport & Fisher (1938) although they are in the same order.

The values for pancreatic extracts are somewhat greater than those for extracts of the small intestine, although both have only a fraction of the activity of the proventriculus. Davenport (1939) has shown that the high carbonic anhydrase activity of the gastric mucosa of rats, cats and

Table 1. *Carbonic anhydrase activities of some tissues of the domestic fowl*

	E. 15° C./mg. fresh tissue.							
Description of bird	Uterus	Uterus serpings	Magnum	Vagina	Blood	Pancreas	Small intestine	Proven- triculus
CH, laying—egg in magnum	0.040	0.076	0.063	0.012	1.07	—	—	—
C1, laying—partly shelled egg in uterus	0.041	0.049	0.020	Trace	1.22	—	—	—
C3, laying—shelled egg in uterus	0.036	0.076	0.032	Nil	1.03	—	—	—
CP, laying—but no eggs in oviduct	0.042	0.108	0.038	Trace?	1.00	0.048	0.026	0.268
DF, had not laid, but was clearly about to lay	0.034	0.042	0.040	0.010	1.28	0.046	0.033	0.645
EP, laying—egg in magnum	0.057	0.094	0.068	Trace	1.12	0.051	0.041	0.422
FP, oviduct and ovary appeared active: had not laid for 10 days. Skeleton depleted	0.029	0.072	0.042	Nil	1.27	0.071	0.041	0.441
Average for laying birds	0.040	0.074	0.043	Nil to trace	1.14	0.054	0.035	0.444
N 2, oviduct inactive and atrophying	0.037	0.069	0.050	Nil	0.92	—	—	—
N 3, oviduct much atrophied	0.013	—	0.009	”	1.37	—	—	—
N 1, oviduct in resting state	0.039	—	0.037	”	1.08	—	—	—
GP, oviduct in resting state. Last egg one week previously	0.022	0.056	0.042	0.006	1.12	0.060	0.036	0.240
HP, ovary quiescent and oviduct somewhat atrophied	0.019	0.013	0.049	Nil	1.25	0.056	0.024	0.174
Average for non-laying birds	0.026	0.046	0.042	Nil to trace	1.15	0.058	0.030	0.212

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rabbits is located chiefly in the parietal cells and has later confirmed this observation for the dog (1939-40). It is probably also true in the case of the fowl.

Among the oviducal tissues, extracts of the vagina had a very low activity; in several cases carbonic anhydrase activity could not be detected. There was no consistent difference between the activities of extracts of the uterus and the magnum, although the uterine values tend to be lower in the case of non-laying birds than in the case of laying birds. When the activities of extracts of scrapings from the internal surface of the uterus are inspected, it will be seen that in every case the values are higher than for either the uterus or magnum. This suggests that the surface cell layers of the uterine wall have a higher carbonic anhydrase activity than the whole tissue of either magnum or uterus, or than pancreatic extracts. This observation is suggestive when considered in the light of Richardson's (1935) observation that it is the uterine epithelium which appears to be most directly concerned in shell secretion.

Repeated attempts failed to detect carbonic anhydrase activity in egg shells, egg white or egg yolk, and it would be of interest to trace the development of carbonic anhydrase activity in the developing egg.

While the present experiments are far from conclusive, the results suggest that the actual calcium-secreting cells of the uterus may have a carbonic anhydrase activity greater than that of other oviducal tissue. This activity might be concerned in facilitating the hydration of CO_2 produced by respiration and the formation of calcium bicarbonate.

SUMMARY

1. Some preliminary observations on the carbonic anhydrase activity of the hen's oviduct are reported.
2. It is suggested that the carbonic anhydrase activity of the uterine epithelium is higher than that of the remaining oviducal tissues, and that this activity may play a part in shell secretion.

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OBSERVATIONS ON THE MINERAL METABOLISM OF PULLETS

VI. THE MOBILIZATION OF BODY CALCIUM FOR SHELL FORMATION

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(With Ten Text-figures)

OBSERVATIONS on the mineral metabolism of the fowl have made it increasingly clear that there are grounds for regarding some degree of mobilization of body calcium for egg-shell formation as a normal feature of reproductive activity in the domestic fowl, a view which has already been adumbrated by Tyler (1940a). The present paper is a general reconsideration of this hypothesis based on the data of a series of mineral metabolism experiments of which the main results have already been published.

It will be convenient first to summarize the known facts for which any such hypothesis must account, and to outline certain inferences which have been drawn from these facts:

(1) When the calcium intake is adequate, most of the calcium secreted in the egg shells over a period of some weeks is derived from the food (Halnan, 1925).

(2) The average *daily* retention of calcium from the food is ordinarily insufficient to cover the calcium in an egg shell (Tyler, 1940a) even when the calcium intake is satisfactory.

Since an egg shell is normally secreted within a period of about 20 hr. (Burmeister *et al.* 1939), it may be inferred that the difference between calcium retention and shell calcium is made up by intermittent drafts on body calcium, these drafts being replaced during the periods when shell formation is suspended.

(3) Even when the calcium intake is very low, pullets will still produce

shelled eggs; however, egg production is not maintained under such circumstances, and within a short period the calcium content of the egg shells decreases and laying stops (Buckner & Martin, 1920; Common, 1932; Edin & Andersson, 1937).

In such cases body calcium has certainly been drawn upon for shell formation, and therefore body calcium is readily available for the production of at least a few egg shells. Edin & Andersson (1937) estimated that 20% of the initial body calcium could be withdrawn in this way before egg production ceased, while Common (1938) found that up to about 24% of the initial body calcium could be withdrawn for shell formation.

(4) When birds produce eggs on a low calcium intake, there is an intermittent increase in phosphorus excretion associated in time with the period of shell formation and therefore with the period of mobilization of body calcium (Common, 1932). This increase in phosphorus excretion frequently results in a heavy negative phosphorus balance for the daily periods in question.

It may be inferred with some plausibility that this extra phosphorus excretion results from the mobilization of skeletal mineral, the calcium liberated being transferred to the shell and phosphate liberated simultaneously from the skeleton being largely excreted. Most of the calcium has to be drawn from the bones, since at least 98-99% of the body calcium of the pullet is in the skeleton (Common, 1938). A certain increase of phosphate excretion during periods of shell formation might reasonably be ascribed to an intermission of calcium phosphate deposition in the bones (McGowan, 1934) or to the break-up of non-diffusible calcium-phosphorus-protein complexes in the blood as suggested by Halnan (1936a). At the same time such arguments do not account convincingly for increases leading to a heavy negative phosphorus balance for the period in question. This heavy phosphate excretion involves increased excretion of ammoniacal nitrogen in the droppings (Common, 1936a), the inference being that it is excreted in part by the urinary route.

(5) When the calcium intake is high, such intermittent increases in phosphorus excretion are not necessarily apparent (Common, 1936a), although Tyler (1940a) frequently found heavy phosphorus excretion even when calcium intake was high. It may be inferred that skeletal calcium phosphates are not being drawn upon to an appreciable degree under these circumstances; this does not necessarily preclude attack on skeletal calcium (Goto, 1918; Tyler, 1940a).

(6) When the ration is adequate in calcium, the Ca : P retention ratio during the pre-laying period is frequently higher than the normal Ca : P ratio of the skeleton as a whole (Common, 1936a). It may be inferred that the increments of bone mineral laid down during the pre-laying period differ in composition from that of the skeleton as a whole, being relatively richer in calcium content.

(7) When the skeleton is subjected to prolonged drafts on its calcium for shell formation as a result of low calcium intake, not only is calcium removed, but the composition of the bone mineral is altered in the sense that heavy draft results in a lower Ca : P ratio of the skeleton (Common, 1938).

It could be inferred from this that drafts fall preferentially on a mineral fraction of high Ca : P ratio, an inference which would fit in with the inference drawn in paragraph (6) above. Such alterations in the composition of the skeletal mineral might also be ascribed to increases in the phosphate of bone; but in two cases where about 24% of the bone calcium had been withdrawn, calculations suggest that the phosphate content of the skeleton had been reduced as well as the calcium content, the estimated Ca : P ratio of the bone mineral lost being higher than the ratio for the entire skeleton.

(8) When the diet is high in calcium carbonate and the pre-laying Ca : P retention ratio is high, then the plasma alkali reserve increases to a high level during the pre-laying period, and, while there is a certain falling off from the peak values after laying begins, the general level remains high during laying (Common, 1940b).

The composition of skeletal mineral is affected by acid-base equilibrium, and hence is related to blood alkali reserve (Goto, 1918; Morgulis, 1931). The facts just mentioned accord with the view that the mineral material deposited during the pre-laying period and also that deposited intermittently between periods of shell formation have a higher Ca : P ratio than the skeleton as a whole, at least the calcium occurs as carbonate.

(9) Not only does the pullet mobilize skeletal reserves of calcium for shell formation when the diet is deficient in calcium, but there is even some evidence that the cumulative calcium balance may in general tend to decline during the early stages of the laying period and regain its initial value only after the lapse of some weeks (Morgan & Mitchell, 1938). Halnan's (1936b) curves for the calcium content of pullets at different ages also suggest a tendency for the calcium content to decline slightly at about 25-30 weeks of age, i.e. at about the period of early egg

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production. The evidence on this aspect of the question is, however, still meagre.

The facts outlined would accord with the view that mobilization of skeletal calcium for shell formation is not merely an outcome of dietary calcium insufficiency, but that some degree of mobilization may be a normal feature of shell formation in the domestic fowl, and that the skeleton may be an important intermediate calcium depot even under optimal dietary conditions.

At this point the actual alterations in the composition of the mineral material of the bones brought about by drafts for shell formation may be considered more closely. The alterations can be related to the magnitude of the total draft on body calcium in the case of certain rations (Common, 1938). The outstanding alterations associated with heavy draft are (a) a lowering of the ratio $\text{Ca} : \text{P}$; (b) a lowering of the ratio carbonate $\text{Ca} : \text{total Ca}$; and (c) an increase in the ratio $\text{Mg} : \text{Ca}$. These changes are most conveniently expressed by using Morgulis's (1931) method of calculating the results of bone analyses. Morgulis calculates the magnesium as $\text{Mg}_2\text{P}_2\text{O}_8$; the phosphorus equivalent of the magnesium is then deducted from the total phosphorus and the calcium equivalent to this residual phosphorus as $\text{Ca}_3\text{P}_2\text{O}_8$ is calculated. The difference between this calcium, which may be designated as Ca_P , and the total calcium is referred to as residual calcium and designated as Ca_R . If the carbonate present is calculated as CaCO_3 , it will usually be found that the carbonate calcium (Ca_C) is less than Ca_R (Morgulis, 1931). The ratio $\text{Ca}_R : \text{Ca}_C$ itself appears to be related to draft on skeletal calcium (Common, 1938). This method of expressing the results of the chemical analyses of bone must not be taken as implying that the molecular species $\text{Ca}_3\text{P}_2\text{O}_8$ or CaCO_3 , or any other, actually exist as such in the bones.

The changes in the bone mineral brought about by drafts for shell formation could be explained on the supposition that the material removed has a ratio $\text{Ca}_R : \text{Ca}_P$ higher than that of the skeleton as a whole; removal of Ca_R alone (Tyler, 1940a) would correspond to an infinitely high $\text{Ca}_R : \text{Ca}_P$ removal ratio. Similarly, the material laid down during the pre-laying period would also have a high ratio $\text{Ca}_R : \text{Ca}_P$, and may correspond to labile bone mineral; the more labile fraction of bone mineral would thus appear to be distinguished by a high $\text{Ca}_R : \text{Ca}_P$ ratio. While this view constitutes the simplest approach to the question, the same changes could result from a transformation of Ca_R to Ca_P during the laying cycle, as pointed out by Tyler (1940a). There might thus be an absolute increase in skeletal phosphate as well as a relative increase.

It will be seen in the subsequent discussion of day-to-day variations in calcium and phosphorus balance during laying that, while absolute increases in body phosphate may occur during periods of draft for shell formation, such increases appear to be relatively small and are possibly only found in individual cases where the bird displays exceptional efficiency of calcium metabolism. It is still possible that some phosphate might be shifted from the tissues to the skeleton, but information on this point appears to be lacking.

Since bone mineral is undoubtedly readily available for shell formation when the calcium intake is low, it is reasonable to suspect that it is also readily available at more satisfactory levels of calcium intake if required, and that the skeleton therefore functions as a readily available depot of calcium for shell formation. Tyler (1940a) has approached this question by assuming that the calcium and phosphorus contents of body tissues other than bone remain relatively constant in short-term day-to-day balance experiments where live-weight changes are small. The calcium and phosphorus exchanges of the body tissues other than the ovary may then be ascribed almost entirely to exchanges in the bones. Starting from this assumption, Tyler applied Morgulis's method of calculating the results of bone analyses to his data for daily mineral balances. He concluded that, for averages over periods of several weeks, the laying hen does not retain much more than about 1 g. Ca per diem from the food, retention (Ca_A) being the difference between the calcium in the food and that in the excreta, and that the shell calcium (Ca_S) does not ordinarily exceed about 2 g. Ca, about one-half of this coming from the food and about one-half from the skeleton. Tyler further concluded that, if the calcium retained from the food (Ca_A) is less than 1 g., then the skeletal loss is not increased but Ca_S is reduced; if Ca_S is less than 2 g. but 1 g. Ca is retained, then there is a saving of skeletal calcium. Tyler points out that in his experiments the intermittent drafts on skeletal calcium appeared to fall principally on the Ca_R of the bones. The changes in Ca_R were positive on 'non-laying days' and negative on 'laying days', whereas Tyler states that the fluctuations of Ca_P showed no definite trend for 'non-laying days' as compared with 'laying days'. Tyler did not define his use of the terms 'laying day' and 'non-laying day'; a laying day might mean a daily period during which oviposition took place or it might mean a daily period of shell formation, meanings which are not necessarily equivalent.

Tyler discusses the day-to-day relations between calcium retained from the food (Ca_A) and calcium withdrawn from the bones ($Ca_P + Ca_R$)

as well as the relations between Ca_P and Ca_R . He finds an inverse relationship between Ca_A and $Ca_P + Ca_R$ and between Ca_P and Ca_R . It should, however, be borne in mind that such relationships could follow algebraically from the method of calculating the Ca_P figures (i.e. from the phosphorus balance) and from the general limits of Ca_S and Ca_A which applied in Tyler's experiments. Since the draft on skeletal calcium ($Ca_P + Ca_R$) is obtained for laying days by the difference between Ca_S and Ca_A , then if Ca_S tends to be fairly constant at about 2 g., a fairly close correlation between Ca_A and $(Ca_P + Ca_R)$ ought to follow. The plotted values for laying days should fall close to the line $Ca_A + (Ca_P + Ca_R) = 2$, as they do in the case of Tyler's birds C 2 and G 2. The plotted values for Tyler's birds S 1 and S 2, which received calcium sulphate, are different in that they are clustered about a line having a distinctly steeper slope than in the cases of birds C 2 and G 2, suggesting that in these two cases Ca_S was to some extent inversely related to Ca_A . Inspection of the data shows that in both cases this inverse relationship may arise from opposing time trends in the two series, Ca_S displaying a rather irregular tendency to fall throughout each experiment and Ca_A a similar tendency to rise. Such trends might or might not be connected with the experimental feeding. Slight indications of a decline in Ca_S with increasing Ca_A have been noticed by the writers in experiments where calcium was given as precipitated calcium phosphate.

On calculating the regression lines of Ca_R on Ca_P , Tyler found an inverse relationship. Here again it may be noted that if $Ca_R + Ca_P = Ca_S - Ca_A =$ about 1 g. for laying days and $Ca_A = Ca_R + Ca_P =$ about 1 g. for non-laying days, then the correlations may be influenced by algebraic relationships because of the method of calculating Ca_P . The regressions calculated by Tyler agree very well with these equations.

In a later paper Tyler (1940b) presents results obtained with a moulting bird during the post-laying period, and again demonstrates a negative regression of Ca_R on Ca_P . In this instance Ca_A was at a lower general level than in the previous experiments with laying birds and also more variable, and the correlation between Ca_P and Ca_R is also distinctly lower. Over a period of some 14 days after the cessation of laying, this bird exhibited a fairly considerable negative phosphorus balance while the calcium balance remained positive. This is an observation of some importance. The Ca : P retention ratio is frequently high during the pre-laying period when the calcium intake is adequate, and a high Ca : P retention ratio has also been observed after the cessation of laying (Common, 1933); but Tyler's data appear to constitute the first record of a significant positive calcium balance associated with a significant

negative phosphorus balance during the post-laying period. Moreover, the losses of phosphorus are somewhat large to be explicable on the grounds of involution of the reproductive system. The diet in this case was supplemented with calcium gluconate and presumably fairly basic in acid-base balance.

Tyler's general conclusion that the mobilization of skeletal bone mineral for shell formation represents a draft which falls predominantly on bone Ca_R is in agreement with the facts and inferences already outlined. In order to test the wider applicability of Tyler's more detailed conclusions as outlined in preceding paragraphs, his method of calculation has now been applied to several series of balance data, the main results of which have already appeared (Common, 1933, 1936a, 1938, 1940a).

When making such calculations of the results of 'balance' experiments it is of primary importance to relate each Ca_S figure as closely as possible to the appropriate Ca_A and Ca_P figures, a point whose significance will be obvious from the discussion above. For the purposes of the present paper, therefore, the term 'laying day' does not necessarily connote a day of oviposition but means the daily period during which the major portion of the secretion of the shell in question would normally have taken place. This relation of each Ca_S figure to its appropriate daily balance was simplified because the times of oviposition were nearly all known to within ± 2 hr., and it was assumed that shell formation normally occupies about 20 hr. In by far the greater number of cases the laying day in this sense was the daily period preceding the day of oviposition; most of the eggs were laid in the morning or forenoon, or if later in the day were part of a clutch of three or more eggs. It may be emphasized that correct temporal relation of the data is a major difficulty in the interpretation of all such experiments.

So long as food intake is reasonably steady from day to day, the droppings for a given daily period may be related to the food intake for that day without sensible error, since the rate of passage of food through the alimentary tract is rapid in the laying fowl (Kaupp & Ivey, 1930). Experience with daily mineral balance experiments tends to justify this procedure. For example, if calcium carbonate is introduced into the ration on a given morning with the object of reducing the apparent digestibility of its phytic acid phosphorus, then the amount of phytic acid in the droppings collected on the following morning is sharply increased over that for previous periods, and the apparent digestibility calculated for this first balance day on the foregoing assumption will agree fairly well with the value ascertained over a longer period, provided

violent fluctuations in food intake do not occur (unpublished observation). This would not be the case unless the rate of food passage is sufficiently rapid to justify the procedure adopted.

The phosphorus content of each egg was spread evenly over the balances for the 7 days preceding the laying day, since practically all the phosphorus of the egg passes into the yolk during a period of some 7 days before ovulation (Halnan, 1936b; Warren & Conrad, 1939) and the shell phosphorus is relatively small. The calcium content of the egg apart from the shell was worked into the balance in the same way as was the egg phosphorus; where the actual figure was not known the approximate value was estimated from the average composition of eggs (Grossfeld, 1938) and Ca_A obtained by difference. The magnesium exchanges were neglected in making the calculations because the gains or losses of magnesium by the body during laying are very small relative to the calcium and phosphorus exchanges; the slightly increased retention of magnesium during laying can be accounted for by the magnesium content of the eggs (unpublished observations).

The average results for the laying periods in twenty-six balance experiments as calculated in this way are set out in Table 1 and certain relations between the various average data are illustrated in Figs. 1-5.

The areas of the dots in these figures are proportional to the number of daily observations included in the average in question. The average values from Tyler's experiments are also included in the graphs, being marked by a cross, but the degree to which these values are strictly comparable with those set out in Table 1 is contingent on the interpretation to be placed on the terms 'laying day' with 'non-laying day' in the case of Tyler's data.

It is apparent from Table 1 that there is considerable individual variation among birds with regard to their capacity for assimilating calcium, even from rations of the same or similar calcium content. When the ration is adequate in calcium and food consumption is satisfactory, individual birds may retain from their food amounts of calcium well in excess of 1 g. per diem. It is also true that other birds may show a poor capacity for calcium retention, although food consumption, calcium intake and health are perfectly satisfactory; such birds are usually the less intense layers.

Table 1 suggests that Ca_A may be greater for laying days than for non-laying days in the case of birds which have a high capacity for calcium assimilation (e.g. pullets C 1, 6, 1, HM 7). In the case of birds whose calcium assimilation is less intense (e.g. pullets 12, 6, 11), Ca_A is

about the same for both laying and non-laying days, as was also the case in Tyler's experiments.

Table 1. *Average data for 26 day-to-day balance experiments calculated as explained in text*

Bird	No. days	Laying days (i.e. days of shell formation)				Non-laying days (i.e. days of no shell formation)				Ration		
		Ca _P	Ca _R	Ca _A	Ca _S	Ca _P	Ca _R	Ca _A	days	No.	Ca %	P %
C 1	14	+ 0.06	- 0.35	+ 1.47	1.76	+ 0.09	+ 1.09	+ 1.18	7	2.21	0.41	
C 2	9	- 0.15	- 0.50	+ 0.74	1.39	- 0.05	+ 0.69	+ 0.64	4	2.21	0.41	
NC 3	6	- 0.77	- 0.50	+ 0.14	1.40	- 0.09	+ 0.20	+ 0.11	5	0.26	0.43	
NC 4	6	- 0.59	- 0.40	+ 0.14	1.13	- 0.03	+ 0.10	+ 0.13	3	0.26	0.43	
S 1	9	- 0.59	- 0.49	+ 0.38	1.47	- 0.05	+ 0.44	+ 0.39	2	0.67	0.43	
S 2	7	- 0.47	- 0.31	+ 0.34	1.11	- 0.02	+ 0.35	+ 0.32	2	0.67	0.43	
NS 3	3	- 0.86	- 0.50	+ 0.15	1.51	+ 0.02	+ 0.14	+ 0.16	6	0.67	0.41	
NS 5	3	- 0.53	- 0.72	+ 0.18	1.49	+ 0.04	+ 0.17	+ 0.20	2	0.67	0.41	
LM 1	6	- 0.53	- 0.79	+ 0.11	1.42	+ 0.26	- 0.13	+ 0.13	9	0.26	0.54	
LM 4	4	- 0.65	- 0.57	+ 0.06	1.27	+ 0.10	- 0.03	+ 0.07	7	0.26	0.54	
HM 6	18	- 0.06	- 0.42	+ 1.19	1.68	+ 0.17	+ 0.87	+ 1.04	9	2.12	0.51	
HM 7	19	- 0.18	- 0.39	+ 1.26	1.83	+ 0.29	+ 0.75	+ 1.04	8	2.12	0.51	
1	14	- 0.20	- 0.56	+ 1.30	2.07	+ 0.04	+ 1.17	+ 1.21	7	1.96	0.54	
1	9	+ 0.03	- 0.84	+ 1.07	1.88	+ 0.66	+ 0.41	+ 1.07	12	1.77	1.42	
3	6	- 0.08	- 0.90	+ 1.18	2.16	+ 0.27	+ 0.60	+ 0.88	7	2.83	1.70	
4	9	- 0.13	- 0.49	+ 1.21	1.83	+ 0.01	+ 0.90	+ 0.92	4	1.96	0.54	
4	5	- 0.43	- 0.80	+ 0.71	1.94	+ 0.24	+ 0.52	+ 0.76	5	1.77	1.42	
5	15	- 0.28	- 0.57	+ 1.24	2.09	- 0.15	+ 1.05	+ 0.90	6	1.96	0.54	
5	14	- 0.04	- 0.64	+ 1.04	1.72	+ 0.59	+ 0.52	+ 1.11	7	1.77	1.42	
6	16	- 0.16	- 0.39	+ 1.36	1.88	- 0.01	+ 1.27	+ 1.26	5	1.96	0.54	
6	4	+ 0.38	- 0.74	+ 1.15	1.52	+ 0.15	+ 0.90	+ 1.05	3	1.77	1.42	
7	3	- 0.36	- 0.74	+ 0.72	1.83	- 0.01	+ 0.24	+ 0.23	4	1.88	0.54	
11	6	- 0.04	- 0.78	+ 1.07	1.89	+ 0.31	+ 0.79	+ 1.10	6	1.88	0.60	
12	5	- 0.03	- 0.58	+ 0.94	1.54	- 0.15	+ 0.89	+ 0.74	3	1.88	0.54	
12	8	- 0.09	- 0.54	+ 1.17	1.80	+ 1.25	+ 0.11	+ 1.37	6	2.22	1.52	
12	4	- 0.03	- 0.54	+ 0.93	1.50	+ 0.20	+ 0.72	+ 0.93	3	2.14	1.57	

Note to Table 1

In preparing Table 1 the data were rechecked against the primary data, errors being found in the published data (Common, 1940a) for pullets 1 and 6 owing to incorrect temporal assignment of eggs. Pullet 1 produced fourteen eggs on ration CR, not fifteen, and nine eggs on ration PR, not ten. Pullet 6 produced sixteen eggs on ration CR, not fifteen, and four eggs on ration PR, not five. The corrected data have been used in the present paper, and the errors do not affect the arguments or conclusions reached in the paper where the uncorrected balance data appear.

Fig. 1 shows the relationship between Ca_A for 'laying days' and Ca_S. It demonstrates a definite tendency for birds on low calcium rations (the low percentage of calcium in the ration being the cause of the low Ca_A figures) to lay eggs with a relatively small amount of shell calcium. This tendency has been noted by many workers in the past, although few have determined Ca_A in their experiments. The shell calcium also falls off rapidly after the first egg or two when the ration is low in calcium.

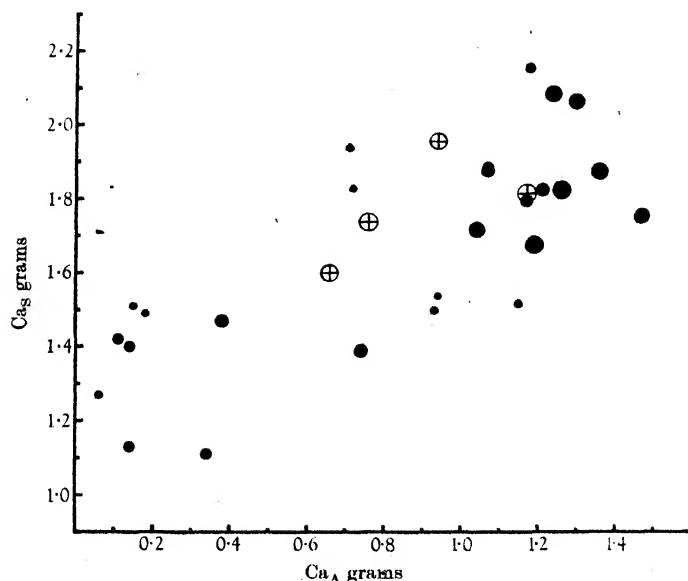


Fig. 1. Relation between average values for Ca_A and Ca_S.

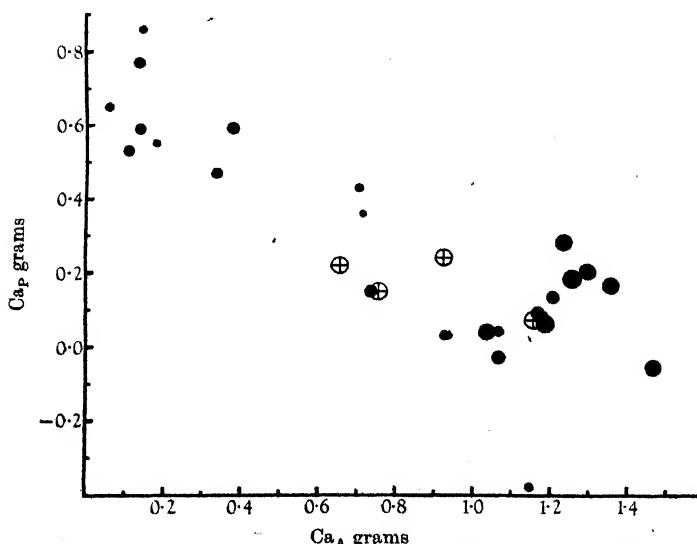


Fig. 2. Relation between average values of Ca_A and of draft on Ca_R for 'laying days'.

The average values for Ca_P for 'laying days' are plotted against the corresponding values of Ca_A in Fig. 2. The most simple interpretation of Fig. 2 is that when Ca_A is less than about 0.7 g. Ca per diem, then drafts on Ca_P begin to become more important. Heavy drafts on Ca_P cannot proceed for long when calcium intake is low without interfering with egg production (Common, 1932; Edin & Andersson, 1937) and it has been suggested (Common, 1938) that this is due to the increasing difficulty which the bird finds in mobilizing skeletal calcium as draft on reserves proceeds. This would accord with the hypothesis that there is a portion of the skeletal mineral material of the laying bird of high Ca : P ratio

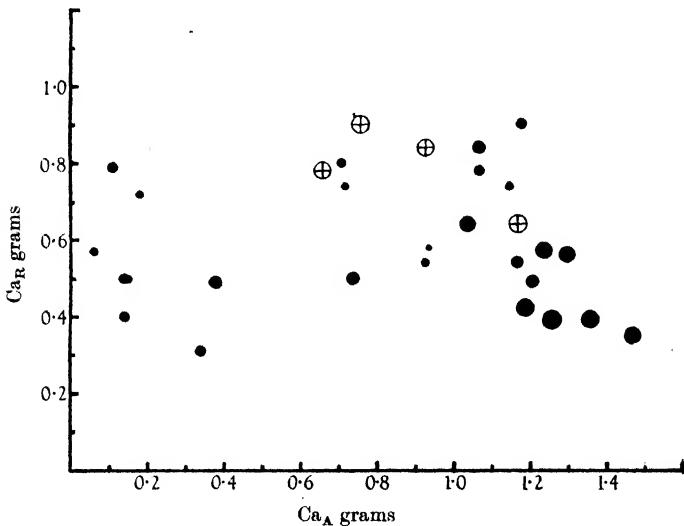


Fig. 3. Relation between average values of Ca_A and of draft on Ca_R for 'laying days'.

which is relatively labile, and that as the Ca : P ratio of the bone material which is being drawn upon declines towards that of the bone as a whole, the difficulty with which skeletal reserves are mobilized increases. This may also be expressed by stating that Ca_R is more readily drawn upon than Ca_P (Tyler, 1940a).

If the drafts on Ca_R are plotted against corresponding values of Ca_A for laying days, as in Fig. 3, there are suggestions that birds which retain more than about 1.1 g. Ca per diem from their food are able to make decreased calls on Ca_R . But when Ca_A is below about 0.5 g., draft on Ca_R does not increase but even displays a tendency to decrease, a feature possibly associated with the low Ca : P retention ratio which

prevails during the pre-laying period on low calcium rations. This contrasts with the tendency for draft on Ca_P always to be higher the lower Ca_A is for the bird in question.

In Fig. 4 the average values for draft on Ca_P are plotted against the average Ca_R values for non-laying days. The results may be separated into two groups, in one of which $Ca_A > 0.72$ g. and the other < 0.72 g.

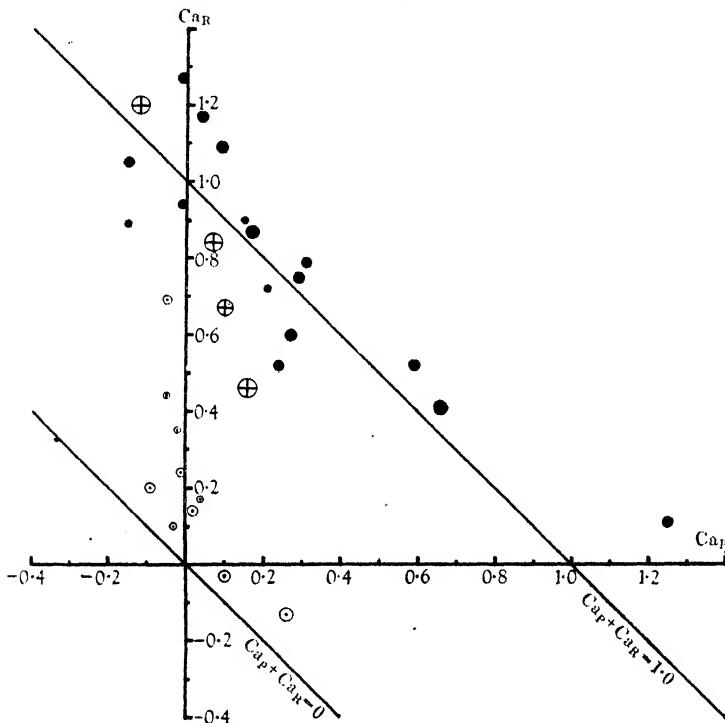


Fig. 4. Relation between average values of Ca_R and Ca_P for 'non-laying days'.
Ordinates are g. Ca_R and abscissae g. Ca_P .

In the case of the latter group, the data for which are marked with circled dots, Ca_A was low because the ration was low in calcium. Since $Ca_P + Ca_R = Ca_A$, and since Ca_A is of the same order in the first group of experiments, the dots fall on a line sloping down from left to right. Very few of the dots are much to the right of the line $Ca_P + Ca_R = 1$ because Ca_A is not very often much greater than 1.0. In the case of the second group of experiments Ca_A is sometimes small, but never negative; hence the dots approach the line $Ca_P + Ca_R = 0$ but never cross it. The figure shows a general inverse relationship between Ca_R and Ca_P at satisfactory

levels of Ca_A (such a relationship being an algebraic necessity if $Ca_A = Ca_R + Ca_P$ = about 1 g. on non-laying days) and there seems to be a fairly wide range of possibilities as to the proportion of Ca_A going to Ca_R and to Ca_P . It is highly probable that the proportions are determined to some extent by the form in which the calcium is given, as will be seen later when day-to-day fluctuations on different rations are considered. At very low levels of Ca_A , there also appears to be a range of possibilities, but it may be noted that for only two birds is Ca_R negative, and the

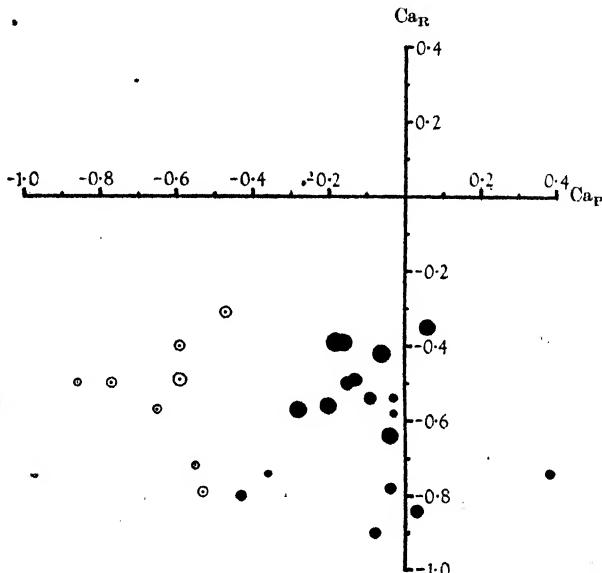


Fig. 5. Relation between average values of Ca_P and Ca_R for 'laying days'.
Abscissae are g. Ca_P and ordinates are g. Ca_R .

distribution of the circled dots illustrates a tendency for birds approaching the satisfactory level to replenish Ca_R even while there is a slight Ca_P loss.

In Fig. 5, Ca_P is plotted against Ca_R for laying days. There is no obvious tendency here for an inverse relation to appear for the averages; the graph merely emphasizes the fact that the ratio $Ca : P$ of the mineral removed decreases for low levels of Ca_A .

When day-to-day fluctuations in Ca_P and Ca_R for individual birds are considered, the direction of the fluctuations in Ca_P as well as of Ca_R are seen to be very definitely related to laying days so long as Ca_A is below about 0.5-0.7 g. per diem. This may be seen from Figs. 6 and 7, which

set out the day-to-day fluctuations in the cases of NC 3 and NC 4 respectively. It is clear from these figures that the drafts on Ca_P and Ca_R for shell formation parallel each other closely, and that the draft on

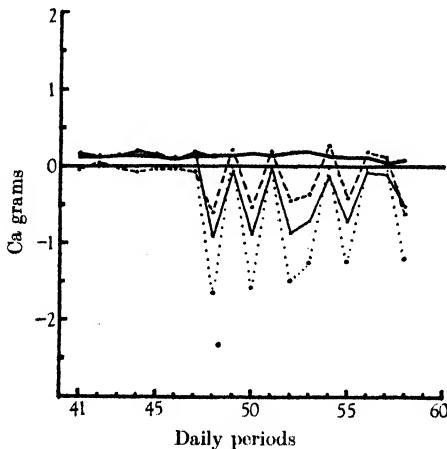


Fig. 6. Pullet NC 3. Heavy continuous line, Ca_A . Light continuous line, Ca_P . Broken line, Ca_R . Dotted line, Ca_S .

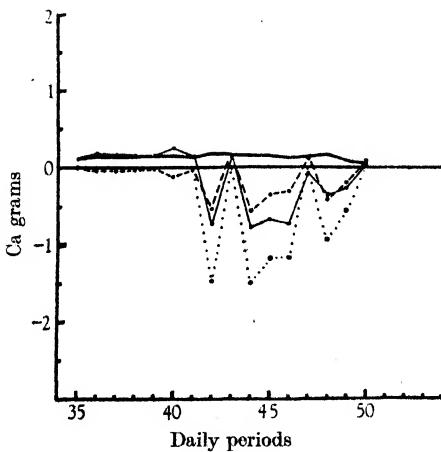


Fig. 7. Pullet NC 4. For key, see legend to Fig. 6.

Ca_R usually exceeds the draft on Ca_P . In the case of both birds the total draft and also the magnitude of Ca_S decrease with time. Thus the difficulty with which bone mineral is mobilized appears to increase as the $\text{Ca} : \text{P}$ ratio of the material removed decreases.

These results are typical of the experiments carried out with rations low in calcium, and it was such results which made evident the capacity of the pullet for making *rapid* calls on skeletal calcium for shell formation. As already pointed out, bone analyses give support to the view that the mineral thus removed from the skeleton has a higher Ca : P ratio than the skeleton as a whole.

Figs. 8 and 9, which relate to pullets 5 and 1 respectively, present day-to-day figures which are representative of the experiments with rations high in calcium. When the calcium supplement is in the form of calcium carbonate, the drafts on Ca_R are closely related to the periods of shell formation and there is storage of Ca_R on days of no shell formation. This is in full accord with Tyler's (1940a) observations. The same things hold for the fluctuations in Ca_R when the supplement is in the form of calcium phosphate, although the storage of Ca_R on non-laying days is at a lower level than in the case of the previous ration. On inspecting the fluctuations in Ca_P , it might at first glance appear that these are unrelated to the fluctuations in Ca_R in the case of the ration supplemented with calcium carbonate. Closer inspection reveals a tendency for a draft on Ca_P to be associated with the second and third eggs of a clutch, a clutch being here taken to signify a series of two or more eggs laid on successive days. Tyler's data also furnish similar indications, for in most cases where his birds laid two or more eggs in successive daily periods, the value of Ca_P is distinctly lower in the case of the second daily period. In short, the Ca_P curve, although less regular than the Ca_R curve, does follow the Ca_R curve as in the case of low calcium rations, but to a less pronounced degree and with a distinct lag on the downward limb of its minimal inflexions. This may be explained on the view that the Ca : P ratio of the mineral mobilized is much higher than in the case of pullets on rations low in calcium, but tends to decrease as the draft increases in consequence of the laying of the successive eggs of a clutch. The presence of this lag explains why, in cases where the clutch consists of one egg only, the Ca_P curve may not follow the Ca_R curve at all, Ca_R alone being mobilized. These effects may also be detected in other similar data relating to the experiments now discussed and also in Tyler's (1940a) data.

When the calcium supplement is in the form of calcium phosphate, the parallelism of the Ca_R and Ca_P curves is clearer than when the supplement is in the form of calcium carbonate (see Figs. 8 and 9). That the lag effect may still be in evidence can be seen in the case of pullet 5; in the case of pullet 1 there is no draft *sensu strictu* on Ca_P , although Ca_P retention is decreased, calcium which would otherwise have been fixed

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in the bones along with some phosphate possibly being deflected to shell formation.

In Fig. 8 the curves follow anomalous courses in the case of the

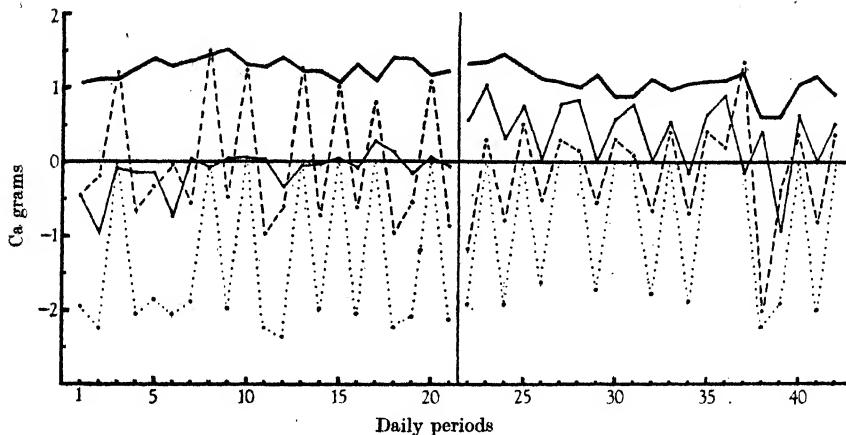


Fig. 8. Pullet 1. Daily periods 1 to 21, supplement of calcium carbonate. Daily periods 22 to 42, supplement of precipitated calcium phosphate. For key, see legend to Fig. 6.

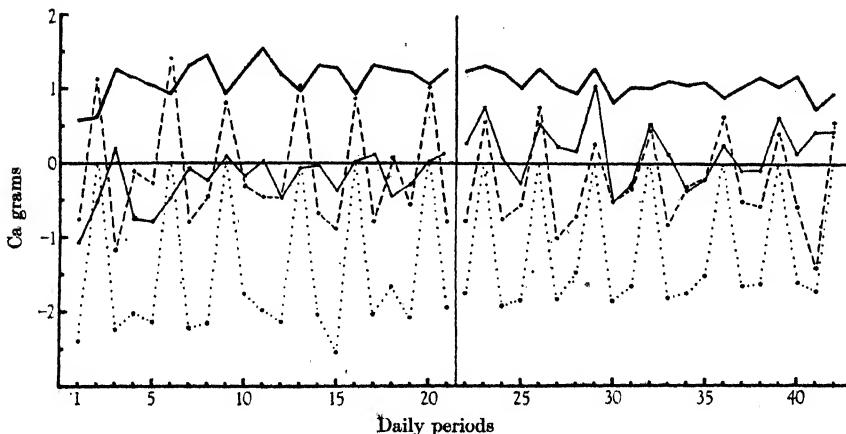


Fig. 9. Pullet 5. Daily periods 1 to 21, supplement of calcium carbonate. Daily periods 22 to 42, supplement of precipitated calcium phosphate. For key, see legend to Fig. 6.

twenty-first egg; it is possible that this egg was retained in the uterus for an abnormal period, and the anomaly disappears if the time of oviposition is advanced by one day.

In short, Figs. 6-9 agree with the view that the ratio Ca:P or

$\text{Ca}_R : \text{Ca}_P$ of the bone mineral mobilized for shell formation tends (1) to decrease as the mobilization becomes more urgent because of the laying of successive eggs in a clutch; (2) to decrease with the calcium content of the ration; and (3) to be higher for a ration supplemented with calcium carbonate than for one supplemented with calcium phosphate.

Figs. 8 and 9 are of further interest in so far as they illustrate the tendency for calcium retention to be higher on laying days than on non-laying days. This relation is very clearly seen in the first section of the Ca_A curve for pullet 5; it is not nearly so pronounced in the corresponding curve for pullet 1, although both birds displayed a satisfactory calcium

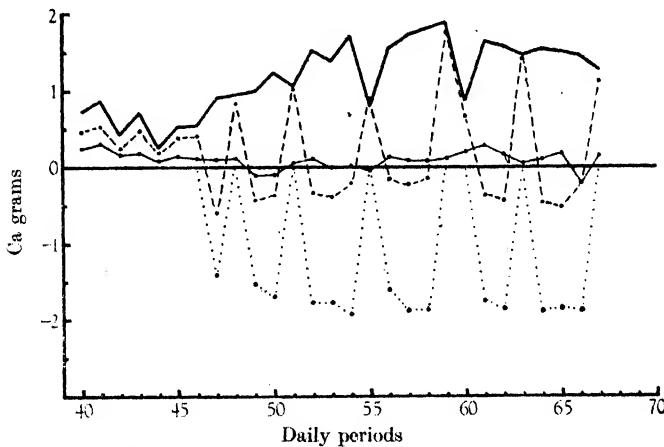


Fig. 10. Pullet C1. Supplement of calcium carbonate.
For key, see legend to Fig. 6.

retention. The existence of this tendency may be missed if data for a limited number of birds are examined.

The day-to-day data for pullet C1 are set out in Fig. 10, because this bird is of special interest on account of its exceptionally high average Ca_A figure. The calcium supplement was in the form of calcium carbonate, and the curves agree in most of their main features with the similar curves already discussed. The upward inflexions of the Ca_A curve for laying days are clear. Laying days are marked by a draft on Ca_R , but owing to the high Ca_A figures the drafts are relatively somewhat lower than in the cases of pullets 1 and 5. Ca_S tends to increase to a steady level of about 1.9 g. calcium, and so excellent was this bird's performance in respect to calcium metabolism that, in spite of the laying of several clutches, Ca_P appeared to be comparatively unaffected by laying. Indeed, pullet C1 stored a little phosphorus on the greater number of her laying days. This

fact is of interest in connexion with Tyler's view that a bird may lose Ca_R while gaining Ca_P , especially since the live weight of this bird was very steady over the period in question, so that significant increases in tissues other than bone were unlikely. Pullet C 1 was in positive calcium and phosphorus balance for the laying period as a whole, gaining 4.20 g. calcium and 1.44 g. phosphorus. The material gained by the skeleton during the laying period therefore had a Ca : P ratio of at least 2.82, so that the gains of Ca_P would not appear to have involved a decrease in the Ca : P ratio of the skeleton as a whole below that at the onset of laying. If such a decrease had taken place, this would have militated against the general applicability of the view that the Ca : P ratio of the skeleton is related to the magnitude of the draft on body calcium, more especially as the calcium supplement in this case was in the normal form of calcium carbonate. (Evidence with regard to the comparative effects of calcium supplements in different forms on bone composition in conjunction with the effects of drafts on skeletal calcium is still very scanty.) The higher storage of Ca_R than of Ca_P during the pre-laying period is also to be noted, although a steady pre-laying increase of Ca_A was upset in the case of this bird by a passing interruption of appetite.

The evidence presented in these curves suggests that the draft on Ca_R during a given daily period is normally limited, and that as Ca_R attains greater negative values, Ca_P is called upon to an increasing extent. In other words the Ca : P ratio of the material withdrawn, when plotted against $Ca_P + Ca_R$, might be expected to follow a curve which approaches asymptotically to an axis passing through the Ca : P ratio for the skeleton as a whole. The interpretation of such changes on a mathematical basis may be complex on account of the many factors which must be considered and which are affected by changes in the calcium and phosphorus intake; the chemical composition of bone mineral which is being rapidly dissolved and redissolved and its equilibrium with the *milieu intérieur* involve the consideration of adsorption equilibria and the metabolic activity of the bone cells as well as of solubility products (Logan & Taylor, 1937).

The foregoing discussion fails to take account of Tyler's (1940a) view that 'if the value of Ca_A is below 1 g. then part goes to Ca_P and part to Ca_R , but if the value of Ca_R exceeds 1 g. then there is a loss of Ca_P to keep the value of Ca_A within its limits'. This view seems to imply that when large amounts of calcium (i.e. much greater than 1 g.) are being retained, then the bones still take up about 1 g. calcium but a transformation of Ca_P already in the bone to Ca_R takes place.

While such changes may quite conceivably take place, how far they may be conditioned by the magnitude of the calcium retention and how far by the state of acid-base equilibrium cannot be regarded as at all clear. Tyler's observations on a moulting bird may also be interpreted, on the view that a change of Ca_P to Ca_R was taking place, although in this case the amounts of calcium retained were much smaller than 1 g. per diem. During the post-laying period the bird probably tends to make up losses of skeletal material, and since these losses will have fallen relatively more heavily on the calcium than on the phosphorus of the bones, it is not surprising to find a high $Ca : P$ retention ratio in the post-laying period. It is not so easy, however, to see reason why such a high $Ca : P$ retention ratio for the skeleton during the post-laying period can pass to a negative value by reason of actual loss of phosphorus from the skeleton, unless laying is associated with increased phosphate deposition in the skeleton. Further work is required in order to disentangle the physiological effects due to reproductive activity and effects due to nutritive factors, including the $Ca : P$ ratio and acid-base equilibria. Such factors are known to affect bone composition (Goto, 1918; Marek *et al.* 1934), and hence possibly the composition of the material laid down before and after laying as well as the composition of the material mobilized for shell formation.

The view put forward in the present paper is that the bird normally uses Ca_R as the predominant source of readily available calcium for supplementing resorbed calcium in shell formation, as suggested by Tyler (1940a), but that Ca_P is drawn upon to an increasing extent as the level of Ca_A falls as a result of low calcium intake, and also as the severity of draft increases temporarily by reason of the laying of several eggs on successive days. This view may possibly help to explain some observations on the serum phosphatase of laying birds (Common, 1936b). Normal laying birds receiving a ration of satisfactory calcium content do not necessarily display a large increase in serum phosphatase with the onset of laying, although certain individuals may do so. When the food is low in calcium, very large increases in serum phosphatase are associated with laying. This might mean that when Ca_A is at a satisfactory level and it is Ca_R which is mainly concerned in making up the quota of calcium for Ca_S , then the drafts on bone calcium will not involve abnormally high serum phosphatase activity; when Ca_A is low, then Ca_P is attacked to an increasing extent involving intense phosphatase activity in the skeleton which is reflected in greatly increased serum phosphatase activity. The curious irregularities observed in the serum phosphatase of

normal laying birds under excellent nutritional conditions and on range may be a reflexion of variations in Ca_A due to varying intake of calcareous grit, or possibly to variations in the relative proportions in which individual birds attack Ca_P and Ca_R apart from variations in Ca_A .

Nearly all investigations of the calcium metabolism of the fowl show that the hen's laying performance is closely related to calcium metabolism. It is not yet clear, however, whether a given hen will be a poor layer because her capacity for calcium metabolism is low, or whether her calcium metabolism is primarily conditioned by reproductive activity. Nutritional experiments naturally tend to stress the effects of variations in calcium supply upon egg production; but physiological experiments often suggest that a poor capacity for calcium metabolism goes hand in hand with a poor laying performance. It may be that each viewpoint supplies part of the whole picture. For example, a deficiency of vitamin D affects laying adversely, and here it is most probably the capacity for calcium metabolism which is primarily affected. On the other hand, the increasing capacity for calcium retention which manifests itself during the pre-laying period suggests that here the awakening activity of the reproductive system is exerting a control upon calcium metabolism. It is an interesting corollary that duration of daylight, which controls the reproductive cycle in the fowl, must also exert a control upon calcium metabolism quite apart from the effect of sunlight as a vitamin D producer. From the practical standpoint, it is obviously of the greatest importance that the laying pullet shall be enabled to build up a sound well-mineralized skeleton during her growth period, and that this skeleton at the onset of laying will have a satisfactory $\text{Ca} : \text{P}$ ratio. This possibly means that the diet ought to have an acid-base balance which will favour the deposition of mineral of high $\text{Ca} : \text{P}$ ratio in the pre-laying period; it is obviously sound policy to provide oyster shell even before laying begins.

Finally, there is one theoretical point of interest which requires serious attention in the future. It is generally held that growth and skeletal development in the fowl are controlled by the anterior pituitary hormone activity, which also controls reproductive activity. Only when growth and bone development are nearly complete does anterior pituitary activity change over from a predominantly growth-regulatory function to a predominantly reproductive function. If the egg were not shelled, this view would fully accord with the facts of calcium metabolism in the fowl, bone growth and bone mineral deposition slowing up and stopping as the ovary comes into activity. But the evidence with regard to calcium metabolism in the fowl suggests that while bone growth may stop with

the onset of ovarian activity and active growth of the ova, the rate of calcium deposition in the bones increases provided the calcium intake is satisfactory. It can reasonably be argued that this pre-laying bone mineral metabolism differs qualitatively in some way from that of growth, especially in view of the high Ca : P ratio of the increments of bone mineral, but the fact remains that bone mineral metabolism seems to increase in activity with the approach of laying activity. The question of hormonal control obtrudes itself again, and the differences in the nature of the calcium metabolism of the bones in the fowl as it enters the reproductive stage suggest that from a more direct control of this metabolism, the anterior pituitary begins to exert a less direct control through the ovaries.

It is feasible on the basis of the observations discussed in the present paper to put forward the following tentative views with regard to the calcium metabolism of shell formation. These views apply more strictly to the case where the calcium is provided in the normal form of calcium carbonate and may require modification as more information becomes available with regard to the effects of other calcium supplements such as calcium phosphate or sulphate, etc.:

(1) With the onset of reproductive activity the physico-chemical equilibria of the pullet's body tend to be modified so as to lead to increased deposition of calcium in the skeleton. Provided the ration contains sufficient calcium (i.e. some amount greater than 'endogenous' calcium excretion plus normal deposition) this tendency will reveal itself by an increasing rate of storage of calcium over a period of 1-2 weeks before the laying of the first egg.

(2) The mineral metabolism deposited in the skeleton during this pre-laying period does not necessarily represent an increment of the same mineral material as is already present. Where the ration is high in calcium carbonate the new mineral will have a Ca : P ratio considerably higher than that of the skeleton as a whole.

(3) The retention of calcium from the food is maintained at a high level during the laying period, and there also exists a tendency for calcium retention to be higher during periods of shell formation than during periods of no shell formation, although this is not necessarily to be detected in all individuals; but even where the ration is high in calcium, the calcium secreted in the egg shell is very rarely completely covered by the calcium absorbed from the food during the period of shell formation.

The difference is made up by a mechanism of recurrent drafts on skeletal calcium. These drafts tend to be replaced again during the

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periods when shell secretion is suspended. All the available evidence supports the view that some degree of mobilization of skeletal calcium is a normal feature of shell formation in the domestic fowl.

When laying begins and the ration is low in calcium, the drafts on skeletal calcium cannot be made up again between laying days. Laying is interfered with and the amount of calcium in the shells falls off. Nevertheless a pullet can use up to one-quarter of her total body calcium for shell formation when the dietary supply is inadequate, in spite of the fact that the skeletal calcium is mobilized with ever increasing difficulty.

(4) The mineral material removed for shell formation does not necessarily have the same composition as the skeleton as a whole; all the evidence supports the view that it has a higher Ca : P ratio than the skeleton as a whole.

Other things being equal, the smaller the total draft on skeletal material, the higher the Ca : P ratio of the material removed. Hence when the ration is high in calcium an excretion of phosphorus from bone does not necessarily accompany the drafts on skeletal calcium. If the ration is low in calcium then the attacks on bone reserves overstep the limits of the normal intermittent drafts, and shell secretion is accompanied by excretion of phosphorus due to heavy drafts on skeletal reserves. If the ration has been poor in calcium during the pre-laying period as well, this effect may be all the greater in the case of even the first eggs laid because the skeleton has not previously received an increment of mineral of high Ca : P ratio. The lower the Ca : P ratio of the material mobilized, the greater the difficulty with which it is removed.

(5) During the post-laying period the calcium retention will fall off again, but the Ca : P retention ratio may still be higher for a time than the ratio for normal bone because of the replenishment of a skeleton depleted of material of high Ca : P ratio (Common, 1935). Moulted birds may actually display retention of calcium at the same time as they are losing phosphorus (Tyler, 1940b), and further investigation is required before this fact can be related to the working hypothesis now propounded.

SUMMARY

1. Some recent developments of mineral balance studies on laying fowl are discussed and applied to the interpretation of the average results of twenty-six balance experiments with pullets.
2. Several of the experiments are re-examined in detail from the same standpoint.

3. A tentative hypothesis covering the relations between the calcium metabolism of shell formation and the calcium-phosphorus metabolism of bone is put forward on the basis of this reconsideration of available data. It is suggested that some degree of mobilization of skeletal calcium is a normal feature of shell formation in the fowl, the fraction of bone mineral material mobilized always having a higher Ca : P ratio than the skeleton as a whole, although the actual ratio may vary with the calcium in the diet and with the form in which the calcium is provided.

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SOIL STUDIES IN RELATION TO GEOLOGY IN AN AREA IN NORTH-EAST SCOTLAND

PART I. THE MINERALOGY OF THE SOIL PARENT MATERIALS

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(With One Text-figure)

THE parent materials of the cultivated soils of Scotland are in the main glacial drifts, and the complex nature of these deposits has given rise to difficulties in distinguishing soil types. Accounts have already been published by the author (Hart, 1929 *a, b*) of the results of investigation into the mineralogical composition of soils of the south-east of Scotland. The results showed that the mineral composition of the parent materials (glacial drifts) approximated to that of the underlying rock formation. In that area the geology is fairly simple and the rock formations uniform and extensive. The present study is concerned with an area of more complex geology and with the parent materials of the soils.

In Kincardineshire in the north-east of Scotland not only is the solid geology fairly complex but at least three ice movements with three consequent boulder clays have been detected. The glacial geology of this area has been intensively studied in recent years by Bremner (1925, 1928, 1937). The three boulder clays will be referred to as the lower, the middle and the upper respectively. The first was laid down by ice moving from north-west to south-east and the third moved in a direction similar to the first but did not reach the coast in the southern part of the area. It is not to be expected, therefore, that the first and third boulder clays will differ much in composition, save that the third may incorporate material derived from the second. The second or middle boulder clay was laid down by ice moving from south-west to north-east. This ice sheet traversed Strathmore, incorporating in its ground moraine red marls which give a distinctive colour to the boulder clay. In addition to these three a dark shelly boulder clay has been found at various points near the coast (Campbell, 1934) formed by an invasion of the Scandinavian Ice-sheet.

GEOLOGY

The geology of the area is described in Sheets 67 and 77 of the 1 mile to 1 inch maps of the Geological Survey of Scotland. The following rock groups occur:

Old Red Sandstone	(Lower)
Silurian	(Downtonian)
Upper Cambrian (?)	(Highland Border Rocks)
Metamorphic Series	(Schists, gneisses)
Intrusive Igneous Rocks	(Granites, epidiorites)

The first three groups lie to the south of the area and are separated from the last two by the Highland Boundary fault. The Highland Border rocks consist of lavas and altered siliceous sediments—they occur near

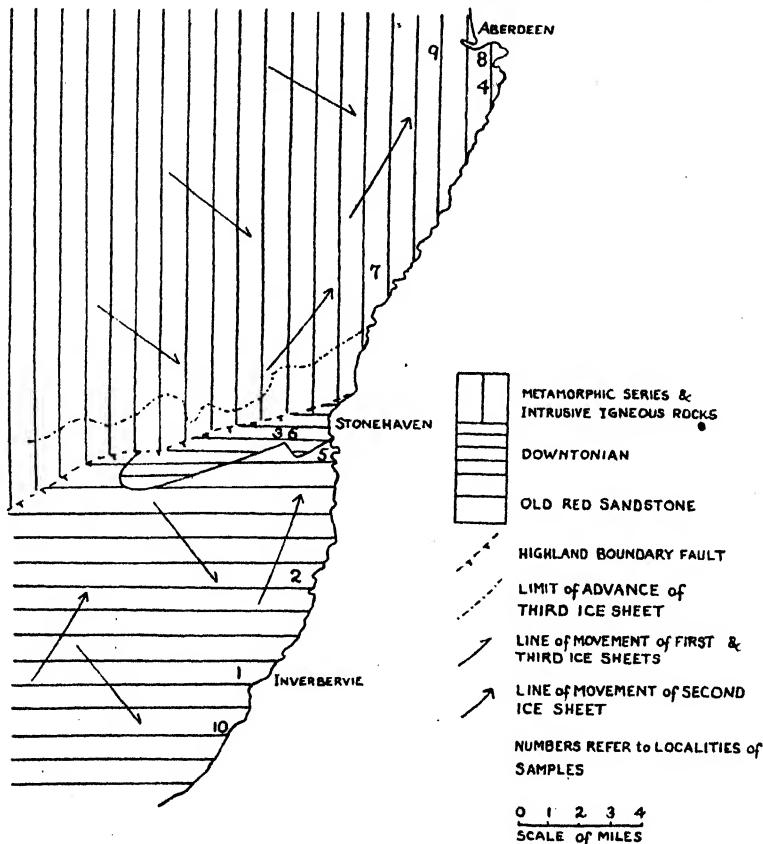


Fig. 1. Map of geology and glaciation of eastern Kincardineshire.

the Highland Boundary fault and are not extensive; while the Downtonian Series is made up mainly of breccias, sandy mudstones and sandstones. These are succeeded by the Old Red Sandstone group, which consists of conglomerates, sandstones and marls, interbedded with lavas and tuffs.

North of the fault lies a series of metamorphic rocks, schists of various types and gneisses. These are intruded by granites and epidiorites.

Overlying all these and forming the parent materials of the soils are the glacial deposits, boulder clays, morainic drift, sands and gravels and also alluvium and peat.

DESCRIPTION OF BOULDER CLAYS

Samples of all four clays were obtained from various parts of the county (see map, Fig. 1), and in Table 1 is given a list of the clays examined and their localities, with a note on the underlying rock formation.

Table 1

Sample	Locality	Description	Underlying rock formation
Lower boulder clay			
1	Inverbervie	Reddish brown boulder clay	Old Red Sandstone
2	Catterline	Reddish brown boulder clay	Old Red Sandstone
3	Mill of Forest	Greyish brown boulder clay	Downtonian
4	Nigg	Grey boulder clay	Metamorphic Series
Middle boulder clay			
5	Stonehaven	Red boulder clay	Old Red Sandstone
6	Mill of Forest	Reddish brown boulder clay	Downtonian
7	Newtonhill	Reddish brown boulder clay	Metamorphic Series
Upper boulder clay			
8	Balnagask	Grey boulder clay	Metamorphic Series
Dark shelly boulder clay			
9	Bridge of Dee	Black shelly boulder clay	Metamorphic Series
10	Gourdon	Black shelly boulder clay	Old Red Sandstone

In the Old Red Sandstone areas the lower clay is reddish brown (as at Inverbervie and Catterline), while north of the fault it is either greyish brown or grey. There is not much variation in the colour of the samples collected of the middle clay: they are generally reddish brown owing to the strong influence of the Strathmore red shales and marls. The middle clay is very extensively developed in the south-eastern part of Kincardineshire. The upper boulder clay is only met with in the northern part of the area and is generally grey brown in colour.

Nos. 9 and 10 clays are peculiar in that they contain arctic shells, are black in colour and do not contain many stones. These clays are very restricted in distribution and are not important agriculturally.

MECHANICAL ANALYSIS

The mechanical analyses of the boulder clays are given in Table 2. The material was air-dried, sieved through a 2 mm. sieve and the analysis carried out on the material passing through the sieve.

Table 2

	1	2	3	4	5	6	7	8	9	10
Coarse sand	22.7	24.9	15.5	27.3	16.7	20.0	21.1	30.7	10.4	12.1
Fine sand	15.7	11.5	21.3	8.2	17.5	15.5	11.4	12.6	17.3	19.0
Silt	35.6	37.3	27.2	32.5	33.5	29.4	14.2	31.0	41.6	50.5
Clay	26.0	26.3	36.0	32.1	32.4	35.2	53.3	25.7	30.7	18.4

All the boulder clays are very stony: in nearly every case the samples contain over 30% of stones and gravel (material > 2 mm.). Only in nos. 9 and 10 was there less than 10%. The clay and silt contents of the lower clays are fairly consistent, while there is much variation in those of the middle clays. In the black clays, nos. 9 and 10, the silt content is noticeably high.

MINERALOGICAL ANALYSES

It has been found from previous experience that the fine sand grade (0.2-0.02 mm.) is most suitable for mineralogical analysis. The separation of the minerals of the fine sand fractions obtained by mechanical analysis into groups according to the density of the minerals was carried out as before (Hart, 1929a), the light group containing quartz and felspars and the heavy group mainly ferromagnesian silicate minerals and iron oxides. The heavy liquid used for the separation was bromoform, and an electro-magnet was also used to obtain a clean separation of the mineral groups. The percentage weights of the groups are given in Table 3. It will be noted that the ferromagnesian silicate percentage in practically every case is much higher than that of the boulder clay of south-east Scotland (Hart, 1929a), where the content of the ferromagnesian silicate group only amounted to 8.3%. This is due to the fact that in the north-east the ice has been mainly moving over a belt of metamorphic and igneous rocks, while in the south-east sedimentary formations contributed largely to the glacial deposits.

As would be expected from the composition of the rocks of the area the quartz and felspar groups of the various fine sand fractions greatly predominate. The percentage figures for the ferromagnesian silicate groups are not distinctive enough in themselves to characterize the boulder clays. The variation in the figures for the various groups is due

to the change in mineral composition of the rocks incorporated by the ice and in this complex region a variation is to be expected. For instance, sample 4 from Nigg has a high content of ferromagnesian silicate minerals (20.1%) while sample 3 from Mill of Forest has a relatively low one (10.5%).

Table 3. *Mineral groups in fine sand fractions (per cent)*

Sample no.	Quartz and feldspar group	Ferromagnesian silicate group
Lower boulder clay		
1	86.8	13.2
2	84.1	15.9
3	89.5	10.5
4	79.9	20.1
Middle boulder clay		
5	85.5	14.5
6	78.4	21.6
7	81.9	18.1
Upper boulder clay		
8	86.8	13.2
Dark shelly boulder clay		
9	89.7	10.3
10	93.1	6.9

For comparative purposes not only must the percentage figures be taken but also the mineral composition of the groups. Microscopic examination of both groups showed that the minerals, in contrast to those previously studied in soils (Hart, 1929a, b), are marked by relatively large size, most of them approaching the maximum particle size for 'fine sand' (0.2 mm.): their freshness is also noteworthy. The grains are also remarkably angular in all the samples apart from no. 10, where some grains are well rounded, suggesting that they have been water-worn.

In the quartz and feldspar groups quartz predominates, forming, it is estimated, over 80%. The feldspars consist of plagioclase, orthoclase and microcline and are present in all the samples in varying amounts. The plagioclase and orthoclase are usually turbid but the microcline is generally fresh.

In characterising the boulder clays the minerals of the ferromagnesian silicate group are most important and a list of these is given in Table 4, where the frequency of occurrence of the minerals (determined by a general inspection of the fractions under the microscope) is indicated by symbols.

Table 4. *Mineral composition of the ferromagnesian silicate groups*

d. = dominant; v.a. = very abundant; a. = abundant; v.c. = very common;
c. = common; s. = scarce.

	Lower boulder clay				Middle boulder clay			Upper boulder clay	Dark shelly boulder clay	
	1	2	3	4	5	6	7		9	10
Iron oxides	d.	d.	v.a.	v.a.	d.	d.	d.	a.	d.	v.a.
Augite	c.	v.c.	—	s.	s.	v.a.	—	—	—	c.
Enstatite	—	—	—	—	s.	—	—	—	—	c.
Hypersthene	s.	—	—	s.	—	c.	—	s.	c.	c.
Diopside	—	s.	—	—	—	s.	—	—	—	s.
Hornblende	a.	v.a.	c.	d.	a.	c.	v.c.	v.c.	v.c.	v.a.
Actinolite	—	s.	s.	s.	—	s.	—	s.	c.	s.
Sillimanite	—	—	s.	c.	s.	—	s.	s.	—	s.
Andalusite	—	—	—	s.	—	—	—	—	—	—
Biotite	c.	a.	d.	v.a.	a.	v.c.	v.a.	d.	v.a.	v.a.
Muscovite	v.c.	a.	d.	a.	v.c.	v.c.	v.a.	v.c.	v.a.	a.
Chloritoid	—	—	s.	—	—	—	—	—	—	—
Epidote	s.	c.	s.	c.	s.	—	s.	c.	s.	v.c.
Zoisite	—	—	s.	s.	—	—	—	—	—	s.
Staurolite	s.	s.	c.	c.	—	s.	s.	s.	c.	c.
Kyanite	s.	s.	—	s.	—	—	s.	—	c.	c.
Apatite	s.	—	—	—	s.	—	—	—	s.	s.
Garnet	v.a.	c.	a.	a.	v.a.	a.	v.c.	a.	v.c.	d.
Zircon	c.	—	—	c.	c.	—	c.	s.	c.	—
Tourmaline	s.	s.	c.	s.	c.	c.	c.	c.	c.	s.
Rutile	—	—	—	—	s.	s.	—	—	—	—
Corundum	—	—	s.	—	—	—	—	—	—	—
Chlorite	s.	s.	—	—	—	—	—	—	—	—

The individual minerals of the ferromagnesian silicate group are briefly described below:

Iron oxides: ilmenite and magnetite are the commonest iron oxides present and are common to all the clays. Limonite and haematite are also present and leucoxene derived from ilmenite has also been noted.

Augite occurs in greenish brown prisms, stout and angular. The ends are generally frayed and the grains slightly decomposed.

Enstatite is uncommon. The grains are colourless, prismatic, with a good cleavage along the length.

Hypersthene is not common. The grains are prismatic with irregular terminations and show the characteristic pleochroism.

Diopside is uncommon. The grains are colourless, prismatic, with a cleavage developed along the length.

Hornblende: brown, greenish brown, green and blue green varieties have been noted. The extinction angle also varies among the different types. The grains are large, very fresh and prismatic.

Actinolite is fairly infrequent. The grains are greyish green in colour, very slightly pleochroic and exhibit frayed ends.

Sillimanite: individual crystals and fibrous aggregates have both been met with. It is greyish with irregular terminations.

Andalusite has also been noted in clay no. 4. It occurs in stout prismatic grains, faintly coloured but with distinct pleochroism.

Biotite and Muscovite are both very common. The biotite occurs as rather large ragged plates, green and brown in colour. Pleochroic haloes are common. Muscovite occurs in rounded colourless plates.

Chloritoid is very infrequent and has only been noted in clay no. 3. It occurs in fairly thick triangular plates, showing the basal cleavage, and a cleavage at right angles to this. The pleochroism is from olive green to green. The grains are rather small.

Epidote is present in nearly all the clays but in varying amounts. It is very common in no. 10. The grains are generally slightly rounded, yellow green in colour and with a fairly good pleochroism. The form is irregular.

Zoisite is very infrequent. The grains are colourless, small, irregular in shape but with a tendency to be prismatic.

Staurolite was noted very frequently. It occurs in fairly large, rather rounded grains, yellow brown in colour and distinctly pleochroic.

Kyanite is uncommon. The grains are large, prismatic with a marked cross cleavage.

Apatite is rather rare. The grains are clear, prismatic with rounded terminations or completely rounded.

Garnet: colourless, pinkish and pinkish brown varieties have all been noted. The grains are large, irregular and occasionally show an imperfect cleavage.

Zircon: the zircons vary in form: complete crystalline types and prisms with rounded terminations have both been noted. The grains are occasionally large and inclusions are common. Zoned varieties are also present.

Tourmaline occurs in brown and pinkish brown grains, prismatic in habit. Broken grains are common.

Rutile is rare. The grains are foxy red in colour, irregular, with prismatic habit.

Corundum occurs in tabular grains, blue in colour.

Chlorite occurs in rounded green grains.

DISCUSSION OF RESULTS

1. The mineral suites of samples 1 and 2 of the lower boulder clay are similar and the relative frequency of the minerals in each is practically identical. The only difference is that in no. 2 there is the occurrence of actinolite and diopside. Both these clays overlie Old Red Sandstone rocks, but no. 2 occurs much nearer the metamorphic rocks than no. 1. The mineral suite is such as could be derived from the underlying rocks. The association of garnet, augite, hypersthene, hornblende is noteworthy.

Mineral suite of sample no. 3 differs from the preceding two in the increase of such minerals as the micas and the presence of sillimanite, chloritoid, zoisite and corundum and the non-occurrence of augite. The boulder clay overlies Downtonian rocks but these are not extensive and the metamorphic series from which most of these minerals were derived lies immediately to the north.

Sample no. 4 has also a similar suite to no. 3, but hornblende is much commoner. The minerals of the metamorphic series are also important in this suite. The clay overlies a belt of metamorphic rocks.

The above results indicate a variation in the mineral content of the lower boulder clay according to the rock formations over which the ice has travelled.

2. In the mineral suite of the middle boulder clays, i.e. of boulder clay laid down by the ice moving from the south-west to north-east, there is a resemblance to that of sample no. 1 of the lower boulder clays which occurs in the southern part of the area. As a whole there is a decrease in hornblende and also in minerals such as sillimanite, actinolite, diopside and kyanite.

Nos. 5 and 6 overlie rocks of Old Red Sandstone and Downtonian age, and the mineral suite is what would be expected, minerals such as sillimanite probably being incorporated from the lower clay. The presence of augite is a noteworthy feature of these clays. In no. 7 there is a marked increase, compared with nos. 5 and 6, of the metamorphic minerals, augite being absent. The clay here overlies the metamorphic series. Rutile has been recorded only from this middle group.

The middle boulder clay is of special interest because it has been greatly influenced by rocks of Old Red Sandstone age. But in the Kincardineshire area the ice movement responsible for this drift was superimposed on the earlier movement from the schistose area to the

north-west and, because of this, incorporation of the earlier drift has taken place. The proportion of ferromagnesian silicate minerals, therefore, in the middle boulder clays is much higher than in glacial drifts examined from Old Red Sandstone areas in other parts of Scotland. The average percentage for the ferromagnesian silicate group in the middle boulder clays in Kincardineshire is 18.1. Hendrick & Newlands (1923, 1925) in studying the mineral composition of soils from various parts of the country found the ferromagnesian silicate content ranging from 2.3 to 9.7, the latter figure being for a soil in southern Kincardineshire. Hart (1929b) found the average figure for soils derived from glacial drifts mainly from Old Red Sandstone sediments to be 2.3 %, though, in a case where the drift composition was largely influenced by lavas, 8.3 % was recorded for the ferromagnesian silicate fraction. From data by Elder & McCall (1936) in a study of soils from Ayrshire the average percentage of the ferromagnesian silicate group for soils and subsoils over Lower Old Red Sandstone sediments is 2.1, and in a soil on boulder clay over lavas from the same formation the percentage is 1.6. Even allowing for the loss in weathering in soil formation these figures show the marked difference between the boulder clays on Old Red Sandstone in Kincardineshire and those so far examined elsewhere in Scotland.

3. The mineral suite from the upper boulder clay shows abundance of micas, garnet and hornblende and the occurrence of such minerals as actinolite, sillimanite and staurolite. The iron oxides are not so abundant as in the other clays. The metamorphic and intrusive igneous rocks have strongly influenced the drift, and there is a similarity to the mineral suite of no. 4 of the lower boulder clay which also overlies rocks of the metamorphic series.

4. Samples nos. 9 and 10 of the dark, shelly boulder clays differ not only in the angularity of the grains, rounded grains being found in no. 10, but they differ also in their mineral suites. No. 10 is characterized by the abundance of garnets and also by the presence of augite, enstatite, diopside, sillimanite and zoisite which have not been recorded from no. 9.

These results show that even in this area of complex glaciation, where several ice movements have occurred, there is a definite connexion between the underlying rock formation and the drifts above them. This connexion may be obscured as in this district by the incorporation of material from earlier drifts in the later drifts. Therefore, in the study of the soil parent materials it is necessary to determine the composition of all the drifts in the region as well as that of the underlying rocks.

SUMMARY

1. An account is given of the distribution of four separate boulder clays, which form the parent materials of the soils of eastern Kincardineshire.
2. The mineral composition of the fine sand fractions of the boulder clays is given.
3. The boulder clays, notwithstanding colour similarities, have been shown by mineralogical examination to be distinct.
4. The middle boulder clay, largely influenced by rocks of Old Red Sandstone age, is shown to have a higher proportion of ferromagnesian silicate minerals than soils from glacial drifts similarly derived elsewhere in Scotland.

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MARSH SPOT OF PEAS: A MANGANESE DEFICIENCY DISEASE

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(With Plate 8)

I. INTRODUCTION

An observation by Pethybridge (1936) that marsh spot occurred on a plot of peas growing alongside oats affected with grey speck disease led him to suggest that marsh spot may also be caused by a deficiency or non-availability of manganese in the soil. He carried out preliminary experiments to test this hypothesis, and in every case applications of manganese sulphate gave some measure of control. Lohnis (1936) also advanced the same hypothesis to account for the occurrence of the disease in Holland, claiming that sound peas contained more manganese than seeds affected with marsh spot. Ovinge (1938) and Lewis (1939) have noted the beneficial effects of fertilizers or sprays containing soluble manganese salts, and Heintze (1938) has shown that there is a significant correlation between the occurrence of the disease and the readily soluble manganese, for all soils examined from Kent. She concluded that marsh spot depends in part on the solubility of soil manganese.

Many writers have stated that no symptoms can be observed on the vegetative parts of plants affected with marsh spot, the disease being detectable only by an examination of the mature pea seed. The present author (1940) therefore suggested that marsh spot might represent a partial deficiency of manganese, since severe deficiency leads to the death of the plant before seed development (Samuel & Piper, 1929). To test this view the effect of regulated amounts of manganese on the growth of peas has been investigated. The water-culture technique has been used, since it is considered that this technique gives the most satisfactory control of the amounts of manganese available to the plant.

Heintze (1938) grew peas in a sand-bentonite culture medium and found that, in the absence of added manganese, 12-15% of the seed developed symptoms of marsh spot while, in the presence of this element, none of the seed was affected. The results were stated to be indicative

rather than conclusive and no yields were given. Since no mention was made of any leaf or stem symptoms in the cultures without added manganese, it would appear that these plants were obtaining traces of manganese from the culture medium or the reagents used.

II. EXPERIMENTAL

Pea seedlings which had been germinated on mosquito netting in contact with distilled water were transferred to jars (capacity 3.2 l.) containing the following nutrient solution:

Potassium nitrate	1.0 g./l.
Potassium dihydrogen phosphate	0.5 g./l.
Magnesium sulphate	0.5 g./l.
Sodium chloride	0.1 g./l.
Calcium sulphate	0.5 g./l.
Ferric citrate	0.02 g./l.
Boric acid	0.5 mg./l.
Copper sulphate	equiv. to 0.1 mg. Cu/l.
Zinc sulphate	equiv. to 0.2 mg. Zn/l.
Sodium molybdate	equiv. to 0.02 mg. Mo/l.

Regulated amounts of a solution of manganese sulphate were added so that the following series was obtained:

Mn per litre nil; 5 μ g.; 10 μ g.; 20 μ g.; 500 μ g.

All water used was redistilled from a pyrex glass still (Piper & Oertel, 1941), and all the reagents were from a supply which had been carefully purified from all traces of heavy metals, in connexion with other investigations in progress. Most of these reagents were purified by two recrystallizations from glass-distilled water. The calcium sulphate was prepared by precipitation, by means of redistilled sulphuric acid, from a purified solution of calcium chloride, while the ferric citrate was prepared from recrystallized citric acid and specially prepared ferric chloride. The latter was obtained from ferrous sulphate by sulphide precipitation, conversion to ferric chloride and further purification by ether extraction. The nutrient solution was changed once during the period of the experiment. Transpiration losses were replaced, as often as necessary, with glass-distilled water. Six seedlings were used per jar.

All plants grew normally for the first five weeks, and no differences were noted until 39 days after the seeds had been set to germinate. Growth then became slower in the jars free from manganese, and the

characteristic symptoms of manganese deficiency, already described by Samuel & Piper (1929), appeared at the growing tips of the plants and rapidly increased in severity. A fine network of mottled patches developed on the youngest leaves and brown lesions on the internodes of the stem and tendrils near the growing tip. Within 2-3 weeks new growth ceased completely in these jars although the basal leaves remained green for some weeks.

In the jars containing the smallest amount of added manganese (5 $\mu\text{g.}/\text{l.}$) growth was more vigorous than in the manganese-free jars and no deficiency symptoms appeared until 8 weeks after germination. Growth then ceased at the apices of the stems and characteristic early symptoms of manganese deficiency appeared. In addition to the symptoms already described the edges of the younger leaves showed a pronounced curling backwards so that the upper surface of the leaf was strongly convex. This was in marked contrast to the concave or upward curling brought about by a deficiency of zinc. About this time all the nutrient solutions were changed and the amounts of manganese replenished. The fresh supply of manganese induced new growth which was deep green and healthy. However, within a fortnight the mottled chlorosis again developed on the newly forming leaves and, shortly afterwards, the growing tips died. A few flowers were produced by the new growth but no pods were set.

Growth was normal in the cultures to which 10 $\mu\text{g.}$ of manganese per litre had been added, except for a slight mottling which developed on the upper leaves just before the change of solution. Following this change vigorous new growth was produced, as the result of the new supply of manganese, and the plants flowered moderately freely. However, within 3-4 weeks severe manganese deficiency symptoms appeared at the tips of the stems and the terminal buds died, growth ceasing. Although many flowers were produced and several pods set, only four small and imperfectly developed pods ripened. On examination at harvest all the peas in these pods were found to be severely affected with marsh spot. The peas were small and shrivelled and large dark brown to black lesions were seen on the inner faces of the cotyledons (Pl. 8, fig. 1).

The cultures receiving 20 $\mu\text{g.}$ of manganese per litre made normal growth throughout. The plants flowered freely and produced numerous pods. At no time did any symptoms of manganese deficiency appear on the vegetative parts of the plants. A good yield of ripe seeds was obtained, and when these were split open small rust-coloured to brownish



Fig. 1. Peas grown in nutrient solution containing 10 μg . of manganese per litre, showing severe lesions of marsh spot on the inner faces of the cotyledons.



Fig. 2. Peas grown in nutrient solution containing 20 μg . of manganese per litre, showing lesions of marsh spot on the inner faces of the cotyledons.

black spots were seen in the centre of the flat faces of the cotyledons in many cases (Pl. 8, fig. 2). Of the pea seeds 33% were severely affected with marsh spot, 24% were slightly defective and 43% showed no symptoms.

Cultures to which 500 μg . of manganese were added per litre grew normally and made vigorous healthy growth, reaching a height of 162 cm. The plants flowered freely and gave a heavy yield of seed, none of which was defective.

Mean yields from the different treatments are given in Table 1 which also summarizes the type and severity of the manganese deficiency symptoms.

Table 1. *The influence of manganese on the growth and yield of peas*

Mn per litre	Nil	5 μg .	10 μg .	20 μg .	500 μg .
Mean yield:	g.	g.	g.	g.	g.
Tops	4.3	12.5	18.2	21.9	30.7
Seeds	Nil	Nil	0.4	10.2	13.5
Roots	1.3	4.0	3.7	3.2	3.5
Total	5.6	16.5	22.3	35.3	47.7
Deficiency symptoms:					
First appearance	39 days	55 days	58 days	—	—
General nature	Very severe vegetative symptoms, no flowers	Severe vegetative symptoms, few flowers, no pods	Tops ceased growth, moderate flowers, four pods	No vegeta- tive symptoms, flowered and podded freely	Normal growth
Number of seeds	Nil	Nil	6	68	88
Incidence of marsh spot:		%	%	%	
Severely defective		100	33	—	
Slightly defective	—	—	24	—	
Healthy	—	—	43	100	

III. DISCUSSION

The experimental data presented in Table 1 shows that the total yields obtained increased progressively with increases in the amounts of manganese present in the nutrient solution. The growth in those cultures with the lowest levels of manganese was limited by the small amounts of this element added or present in the seed. As soon as this supply was exhausted growth ceased and characteristic deficiency symptoms developed on the plants. The addition of fresh supplies, at the time of the change of the nutrient solution, led to a period of renewed healthy growth. In those cases in which death of the growing tip had already occurred from manganese deficiency, the new growth developed from

buds near the base of the plant. When, however, growth had ceased without actual death of the tips the new supplies of manganese caused the apices of the stems to burst forth with vigorous new growth. Fresh symptoms developed as soon as the additional supplies of manganese were exhausted. Owing to the more vigorous growth rate at this stage, compared with the seedling stage, the added manganese was absorbed more rapidly than the initial amount and deficiency symptoms appeared more quickly. Thus manganese deficiency symptoms reappeared in the cultures receiving 5 μg . of manganese per litre 14 days after the addition of the second lot of manganese (at the change of solution), whereas the initial symptoms did not appear until 48 days after placing the seedlings in the nutrient solution. Thus it is evident that at this stage of growth there is a demand for greater amounts of manganese than in the early seedling stage.

One limitation of the water-culture technique is that it is difficult to maintain an infinitesimally small concentration of the element under investigation. Supplies are rapidly absorbed after each addition and a period of complete deficiency ensues. In a deficient soil it is probable that extremely small traces, sufficient to support a limited amount of growth, are slowly and continuously being made available, and that deficiency symptoms develop on the plant when the demand, due to an increased growth rate, becomes greater than the soil can furnish. According to Heintze (1938) marsh spot develops late in the maturity of the plant. It would thus appear probable that soils subject to marsh spot are able to supply small amounts of manganese, even sufficient to meet the vegetative requirements of the plant when it is growing actively, but not sufficient to enable it to build up reserves or to supply the full amount necessary at the time of seed formation. A deficiency at this stage expresses itself in the lesions occurring on the cotyledons and sometimes on the plumule of the seed.

IV. SUMMARY

By growing peas in water cultures with carefully regulated amounts of manganese, the effects of a deficiency of this element have been studied.

Complete absence of manganese produces a mottling of the younger leaves and death of the growing tip as previously reported. The plant does not reach the flowering stage.

Small amounts of manganese, insufficient for normal requirements,

enable increased growth and seed formation. The seeds show marsh spot lesions, the severity of the lesions being greater at the lower manganese concentrations.

Normal growth and sound seed are produced when sufficient manganese is present in the nutrient solution.

Marsh spot results from a partial deficiency of manganese, the amount available to the plant being sufficient for its normal vegetative requirements but not for healthy seed production.

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LABORATORY EXPERIMENTS ON EVAPORATION FROM FALLOW SOIL

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(With Three Text-figures)

PREVIOUS field studies (Penman, 1940a; Penman & Schofield, 1941) have held out the promise that evaporation rates might be physically interpreted in terms of normal meteorological data supplemented by certain soil data, of which the chief is the value of the water vapour pressure at the soil surface. While the surface is moist it is sufficient to know the surface temperature; when the surface is dry there is no simple way of obtaining the required information. Pending the development of a suitable method there is much semi-empirical information about evaporation processes that remains to be found and the experiments described below are an attempt to fill in some of the gaps in our knowledge. There are three main groups: (1) Evaporation under isothermal conditions, in which, apart from surface cooling produced by evaporation, the soil is kept at air temperature. (2) Evaporation under simulated summer field conditions in which the soil surface is maintained at a higher temperature than the air for part of the day. (3) The effect of dissolved salt in the soil water is studied under both isothermal and non-isothermal conditions.

GENERAL EXPERIMENTAL DETAILS

The experiments were carried out in a constant temperature room, ventilated from outside the laboratory and thus of variable humidity. A constant-speed ceiling fan provided a steady breeze estimated at about 9 miles/hr. over the small area employed in the experiments. Two types of soil have been used in 12 in. cylinders freely drained. Rothamsted allotment soil—a clay loam—was taken in its field condition in Spring, being transferred to the cylinders a little at a time with tamping down after every fresh addition. Woburn soil—a sandy soil—was packed in an air dry condition, with similar small additions and thorough tamping after each. As the cylinders varied in diameter from 5.2 cm. (Woburn soil) to 10.0 and 11.2 cm. (Rothamsted soil) a correction for differences

was made from $E_1/E_0 = (d_1/d_0)^{1.66}$ (Powell, 1940), the standard diameter being that of an open-water surface ($d_0 = 9.6$ cm.). The measured evaporation from open water integrates the effects of air factors (wind speed, relative humidity and temperature), and thus permits a closer study of the effects of soil factors and of radiation on the evaporation process. The open water evaporation also provides a time scale. When needed, surface heating was produced by a 750 W. electric radiator suspended about 2 ft. above the soil surface; the open-water conditions were isothermal throughout.

The general scheme of the experiments is similar to those of Buckingham (1907) but with important changes in detail. There is here no water table maintained at 4 ft., the heating of the surface by radiation is an improvement on his heating band round the top 2 in. of metal cylinders, and the open water surface control is used here in a way which makes the study much more quantitative than Buckingham found possible. In group (2) experiments the radiator was on for about 8 hr. a day and its effect was to raise the surface temperature of dry soil about 10° C. above air temperature. This figure was chosen after making field measurements of soil surface and air temperatures during a week of cloudless anti-cyclonic weather in June 1940. Soil evaporation losses were obtained by weighing the cylinders once or twice daily, to the nearest $\frac{1}{2}$ g. (in 5000 for Rothamsted soil, in 3000 for Woburn soil). Open-water losses were similarly measured to the nearest $\frac{1}{2}$ c.c., the normal daily evaporation being about 40 c.c. although extremes ranged from 1 to 150 c.c./day. For presentation all readings have been converted to inches of water. Thermometers with their bulbs either just below the water surface, or pressed into the soil surface, gave a mean value of the temperatures of surface layers; the wet-bulb temperature of the air was also measured.

EXPERIMENTAL RESULTS

Evaporation under isothermal conditions (Fig. 1). A wide range of drying conditions was obtained by varying air temperature, fan-speed and humidity. The lowest rate of 1 c.c./day (not represented graphically) was obtained by covering the tops of the Rothamsted soil cylinder and the open water tray with lids pierced by three holes c. $\frac{3}{16}$ in. diameter. The curves fall into two distinct groups. (1) The upper group is characterized by an extensive range of approximately unit slope. For each of the soils and for a drying power range of up to 0.22 in. per day (mean air temperature $\leq 20^{\circ}$ C.) the evaporation from the soil is equal to that from

the open water up to about 1 in., at which a slow decrease in the relative rate is apparent. This change in drying rate coincides with the first visual evidence of surface drying, although shrinkage was apparent at an earlier stage in the Rothamsted soil. The results of the slow evaporation experiment on Rothamsted soil are, for various reasons, not so confidently reduced to absolute values, but the relative rates during the 5 months of the experiment show the same steady evaporation rate up to about 0.8 in. lost, a slow decrease in the rate again becoming apparent at about 1.0 in. If one makes the reasonable assumption that the initial slope is near unity, then the experimental points up to 1.2 in. lost lie on or near

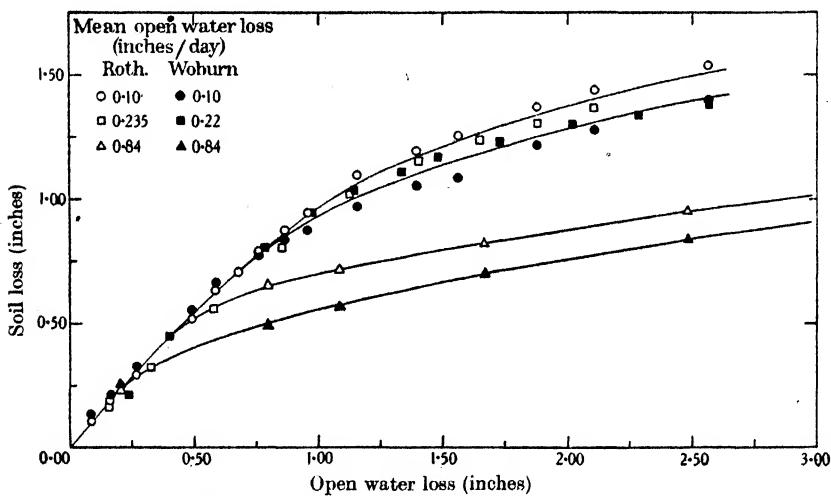


Fig. 1. Evaporation under isothermal conditions.

the mean Rothamsted curve for rates up to 0.22 in./day. (2) In the lower group, obtained with a very great drying power (mean air temperature = 35° C.), the section of unit slope is very short. Surface drying appeared almost complete after 8 hr., the total water loss being then about $\frac{1}{4}$ in.

In both groups the initial drying rate for the sandy soil is greater than for the clay loam, but this order is soon reversed, and eventually the curves run approximately parallel, not only within groups but also between groups.

Conclusions from 'isothermal' experiments. (a) A steady demand on the soil's water supply can be met for a considerable time. The total amount so drawn off is not affected by quite large changes in the rate of extraction.

(b) A very severe demand cannot be steadily met.

(c) The extent of (a) indicates a considerable amount of liquid movement to the surface in spite of the absence of a nearby water-table.

(d) An open water loss of 0.10 in./day corresponds to average English June meteorological conditions. The soil loss in 10 days was 0.94 in., the surface being still dark and moist. This quantity is appreciably more than evaporates from fallow field soil in the same time, and under normal June conditions the soil surface does not remain dark and moist for more than two or three days after rain.

(e) The liquid movement of (c) appears to have a limiting velocity which prevents it from keeping pace with an extremely rapid rate of withdrawal of moisture. Under the conditions of the lower experiments the soils could be described as self-mulched.

Evaporation under simulated summer conditions (Fig. 2). One cylinder of each soil was radiated for 8 hr. a day and kept under steady conditions for the remainder. During the first 2 days weighings were made at the beginning and end of the radiation period but thereafter only one weighing per day was made. Another pair of cylinders was mulched to a depth of c. 1 in. at the beginning of the experiment (before the soil was really in a fit state for this operation). The figure includes the isothermal curves from Fig. 1 for corresponding air conditions.

Before drawing conclusions, certain reservations must be made. Experiments of this type are necessarily qualitative since there are no real standard conditions, and, in the case of the mulching experiments, no reproducibility of conditions. It is thought that the radiation experiment does faithfully simulate certain summer conditions under which possible control of surface evaporation is of technical importance, but the mulching experiments are much less satisfactory. Apart from varying the depth of mulching and the epoch at which it is carried out, they involve a considerable change in the surface geometry, a change that is large in comparison with the size of the surface. The turbulence thereby introduced into the moving air means that the open-water evaporation ceases to be a measure of the drying power of the air passing over the mulched surface.

In a third set of experiments, not represented diagrammatically, mulched cylinders were radiated, the mulching being carried out within an hour or two of the beginning of the first radiation period. For Woburn soil the later portion of the 'mulched and radiated' curve lay above the 'radiated' curve, while for Rothamsted soil it lay slightly below the 'radiated' curve. *For the conditions of the present experiments we can say*

that while there are differences in the early days, after the equivalent of 25 average June days (i.e. 2.50 in. evaporated from open water) the conservation effected by mulching is inappreciable, whether it be impressed on isothermal or radiated conditions.

For the remainder of this section attention is directed to the intermittently radiated soils. No significance can be attached to the differences between the Rothamsted and Woburn curves as removal and replacement of the cylinders for weighing probably involved day to day changes in the relative intensities of radiation falling on the surfaces. The results

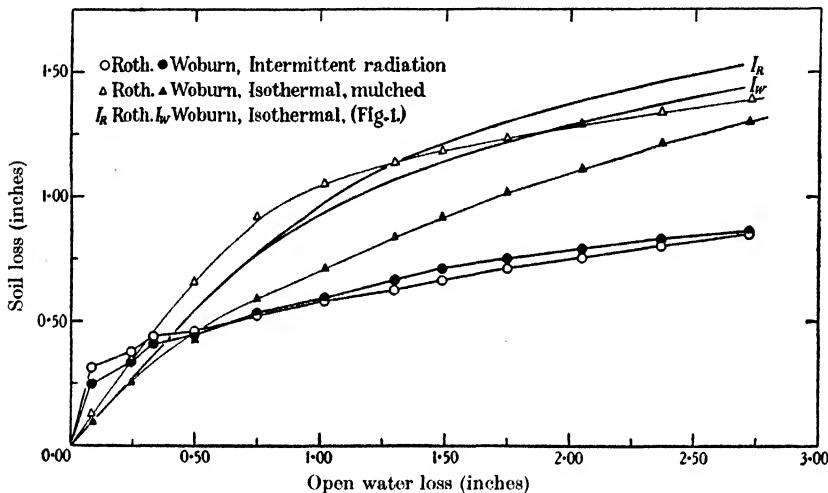


Fig. 2. Evaporation under simulated summer conditions.

show the expected high initial rate of evaporation, a rapid decrease in the rate coinciding with obvious surface drying on the second day, an early intersection with the isothermal curve, and thereafter an almost constant drying rate of the same order as that of the later stages of isothermal drying.

Conclusions from radiation experiments. (a) Rapid initial drying conditions are maintained for only a short period; this period lasts as long as the soil surface is moist, i.e. about 2 days.

(b) Thereafter the rate of loss is nearly constant and there is clear-cut evidence of conservation as compared with isothermal evaporation.

(c) The total loss after the equivalent of 25 June days is c. 0.85 in., of the same order as is found in field experiments. This, with (a), indicates that the intermittent radiation has effectively reproduced summer field behaviour.

(d) Roughly: the total evaporation after t days under radiative conditions is given by $E \approx at^{1/n}$, where $n \approx 3$.

Effect of dissolved salt (Woburn soil) (Fig. 3). Of four cylinders, two were leached with $N/10$ NaCl, the others as usual with tap water. The cylinders, A , B , C and D were treated as follows:

A , Isothermal: salt

C , Radiated: salt

B , Isothermal: no salt

D , Radiated: no salt.

Midway through the first radiation period θ_A —the surface temperature—was greater than θ_B (20.4 , 20.0° C.), and θ_C greater than θ_D (27 , 26),

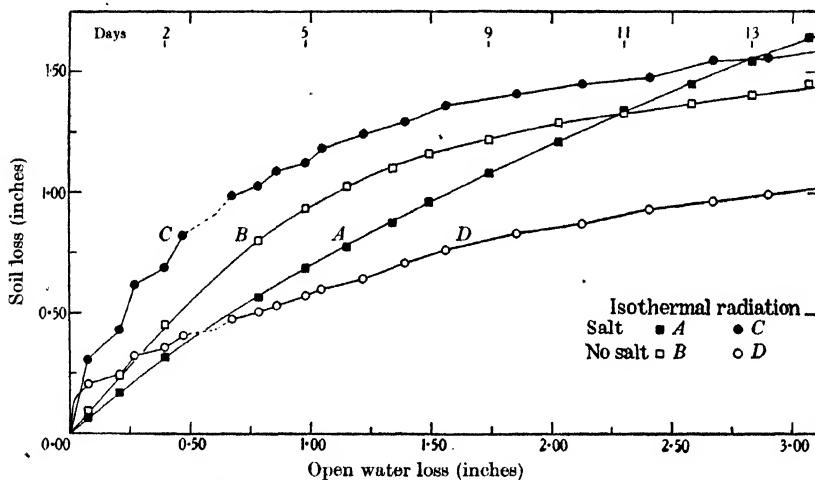


Fig. 3. Effect of dissolved salt on evaporation (Woburn soil).

indicating more rapid evaporation from the surfaces of B and D . At the end of the first radiation period, θ_D was greater than θ_C (30.5 , 29), indicating a change-over in the relative evaporation rates from these radiated surfaces. This evidence justifies the intersection in the curves of C and D between the origin and the first experimental point. Attention is directed to several points of interest.

Initial slopes. As before, we have the general difference between radiated and isothermal conditions, but in each group we see the effect of decrease in vapour pressure due to the dissolved salt.

Subsequent behaviour and intersections. Although the initial 'salt' slopes are less than the others, the reduced rate of evaporation is maintained for a longer period. Thus, while B and D intersect on the second day (cf. Fig. 2), A and C only intersect after 13 days. C and D do not

intersect again nor does *C* intersect *B*; actually in the later stages all three are approximately parallel, showing that under what we may now call normal summer field conditions the evaporation from a saline soil is much greater than from a non-saline soil, and is also greater than from a non-saline soil under isothermal conditions.

Both *A* and *C* showed salt efflorescence after about 10 days, indicating conditions of saturation at the surface. *A* was still dark after 15 days of drying.

Conclusions from salt experiments. (a) The presence of salt in the soil water depresses the initial rate of evaporation, but this decreased rate is maintained for a longer time. Under isothermal conditions one would expect curve *A* to be similar to curve *B* if the open water could have a salt content continuously adjusted to the same concentration as the soil moisture at the surface of cylinder *A*.

(b) Under radiative conditions the supply of liquid to the surface is maintained for a length of time comparable with that for isothermal non-saline conditions. This liquid flow will be of a salt solution and hence a salty patch of soil will (i) continue to evaporate water and remain more moist than neighbouring less salty patches, and (ii) will tend to become saltier at the expense of those neighbouring patches.

Miscellaneous experiments. (1) In each of the figures attention has been drawn to the approximate equality of slope in the later stages. An experiment was performed with Rothamsted soil in which intermittent radiation was maintained until the isothermal curve was intersected; thereafter isothermal conditions were maintained. Compared with an experiment in which intermittent radiation was maintained throughout, the curves ran very nearly coincident and parallel from this point of intersection onward. Thus for a 3-day period the following figures were obtained.

	Mean air temp. ° C.	Mean wet- bulb temp. ° C.	Soil loss g.	Water loss c.c.
Isothermal after intermittent radiation	20.9	17.3	31	122
Intermittent radiation throughout	21.2	17.8	32	130

Ratio of slopes = 1.03.

The cumulative evidence suggests that some equilibrium is eventually attained whatever the nature of the initial behaviour, and that the differences between isothermal conditions, intermittent radiation, and mulching lie in the rapidity with which this equilibrium rate is attained and the gross amount of water lost in attaining it.

(2) The Woburn cylinders used in the salt experiment, and a contemporary Rothamsted isothermal *v.* radiated test were covered up at the end of 15 days drying and left for a fortnight; apart from a slight leakage through imperfect seals there was no further evaporation. The following details were recorded before and after this rest period:

	Cylinder	Appearance	
		2. viii. 40	14. viii. 40
Woburn	A	Dark: efflorescence of needle-like crystals	Dark and moist: no efflorescence
	B	Light	Dark but not obviously moist
	C	Light: (efflorescence blown away)	Darker than B but lighter than A
	D	Light	Approx. same as B
Rothamsted	Glass	Light: cracked: dry down to $\frac{1}{4}$ in. next wall	Dark and some parts moist: no dry layer
	Copper	Light: no cracking but shrunk from wall	Dark: more uniform than glass cylinder

There is evidence of redistribution of moisture here. All surfaces showed a darker appearance, the salt efflorescence disappeared, presumably by re-solution and diffusion away from the surface, and in the only transparent-walled cylinder the dry surface layer was no longer to be seen. The drying was then resumed under isothermal conditions. After 1 day, B, C and D had settled down to a steady drying rate, and in 2 days the others reached a steady rate, the surfaces being light and dry, and in the glass cylinder the dry $\frac{1}{4}$ in. layer was again apparent. An estimate of the amount of water redistributed in the rest period was obtained by subtracting from the total evaporation of the first 2 days the amount evaporating in 2 days at the ultimate steady rate. For the glass cylinder this amount was $28 - (2 \times 5\frac{1}{4}) = 16\frac{1}{2}$ g., i.e. 0.085 in. of water; the evaporation rate before the rest period was 0.045 in./day. Assuming that at the beginning of the rest period the 100% R.H. layer was 2 cm. deep, a rough estimate based on a diffusion equation previously developed (Penman, 1940b) indicates that diffusion of vapour would account for about 95% of this redistribution during the rest period, i.e. it may not be necessary to assume that any appreciable movement as liquid had taken place in spite of the pronounced moisture gradient in the soil.

GENERAL DISCUSSION

Where comparison is possible the results of the preceding experiments are in substantial agreement with those of Buckingham, the absence of a nearby water-table apparently having no effect on the nature of the

phenomena observed. Buckingham suggested that soils would be 'self-mulching' under arid desert conditions, but the present work suggests that this is still true under normal English summer conditions.

The previously observed features of winter and summer evaporation (Penman & Schofield, 1941) are again found in the isothermal and intermittent radiation experiments respectively; that is, for the former, the soil and open water losses are equal for periods of the order of the normal interval between rainfalls, and for the latter, the soil losses are higher in the early stages and less in the later stages than the open water loss. The result of an incomplete survey of the annual cycle of soil surface and air temperatures indicates that conditions may be regarded as isothermal when the mean air temperature is below 48° F., and as non-isothermal above 48° F. For Rothamsted this means that approximate isothermal conditions exist from October to April inclusive, and during the remaining 5 months we must regard field conditions as similar to those of intermittent radiation. Thus the broad difference between winter and summer evaporation is that between isothermal and radiated conditions.

In general, the condition under which mulching has a beneficial effect on water conservation is that specified by Shaw (1929), namely, that the water-table should be within a few feet of the surface. The preceding experiments suggest an alternative criterion, namely that mulching may or may not be effective according to the time of year at which it is done. Thus King (1890-1) found evidence of conservation by a spring mulch, but his results for summer mulching show no significant effect. Veihmeyer's (1927) Californian summer experiments showed no benefit attributable to mulching. He found that the evaporation loss for 80 days was already half complete in 5 days, indicating a relation of the form $E = at^t$, i.e. of the type already suggested for the radiation curves. It is apparent then that during a long rainless period in summer, i.e. at such a time when possible control of evaporation is important, the effect of the sunshine itself is to produce a surface mulch and cultivation does not appreciably affect its efficiency.

There is abundant evidence from the curves that, except under extraordinary circumstances, a steady drain on the soil's water supply can be met, even in the absence of a nearby water-table. The fact that the grass on the Chalk Downs can remain green during a period of drought, although the water-table is some 200 ft. below (Hall, 1904), is a reflection of the ability of the chalk to retain rain water and of the conservative demands made upon the water by the grass. It is no proof that water was moving upward from a water-table 200 ft. below, and those later

writers who have quoted Hall's tentative suggestion as experimental evidence supporting this idea, have done so in spite of the omission of the relevant paragraph from later editions of his book.

Veihmeyer's experiments, and later work, theoretical and experimental, by Schofield (1935) indicate that flow of liquid water from a moist to a contiguous dry soil depends upon the energy gradient, which may be zero even for a positive moisture gradient. The present experiments are, therefore, to be interpreted by assuming that the action of radiation, or of very high air temperature, is to dry out a shallow layer at the surface more quickly than it can be replenished by liquid flow from below. Once this dry layer is produced water movement from below is entirely in the vapour phase, leading to the following consequences:

(a) Evaporation rates are very much reduced. They are only slightly dependent upon air and soil surface conditions; hence the later slopes of all curves are about the same and correspond to a dry layer of about 3-5 mm. thick. The rate is then about $\frac{1}{20}$ in. per day.

(b) When the evaporation process is suspended, the rate of redistribution in the region of steep moisture gradients is almost entirely dependent upon the rate of diffusion of water vapour.

If there is some other factor tending to restrict the effects of the rapid drying power, then evaporation losses may be very great as in the experiments with salt, where the effect of the reduced vapour pressure was to prevent the formation of a dry layer, so that self-mulching action was not apparent at as early a stage as in the non-saline experiments.

The mechanics of the formation of this dry layer is doubtful owing to our limited knowledge of the dynamics of water movement in soils. In the case of surface radiated soil, downward distillation of moisture will undoubtedly help to dry out the surface layer, but this does not seem to be a necessary condition, since in the high air temperature experiment the soil surface was very much cooler than the bulk of the soil, i.e. distillation would take place into, and not away from, the surface layer. The liquid movement depends upon the capillary conductivity and the suction gradient, both being functions of moisture content; the vapour movement depends upon the relative humidity of the soil air and this is not nearly so dependent upon moisture content as the liquid variables are. Hence it is conceivable that while a large decrease in liquid conductance could take place, the vapour conductance would not change appreciably, so that in the thin surface layer the rate of removal by vapour would exceed, and continue to exceed, the rate of renewal by liquid. The condition produced, in which there is a dry layer with ample reserves of moisture

a few mm. away, seems to depend on the existence of a two-fold mechanism for water movement. In the region round a plant's root the plant might produce a similar state of affairs by transpiring so rapidly that it formed a barrier between the roots and the water supply they needed, causing wilting within a short distance of an irrigation channel.

One other point of physical interest deserves mention here. In discussing the isothermal curves we have taken the open water and soil surface losses as equal. In magnitude the slopes are slightly greater than unity but as the temperature of the soil surface was invariably higher than that of the water surface, for the same vapour pressure difference the soil rate of evaporation is slightly *less* than the open water rate. The turbulence introduced by the rims of the containing vessels and by irregularities in the soil surface prevents us from making a precise estimate of the ratio of the rates per unit vapour pressure gradient, but the results of other experiments agree with the implication from these that the ratio is between 0.8 and 0.9 in spite of the fact that only about one-half of the surface is available for the transmission of vapour. This is merely one aspect of a much larger problem of great biological interest in connexion with leaf transpiration and assimilation, namely, how does the rate of diffusion through a perforated plane depend upon the number, size and spacing of the perforations?

SUMMARY

Experiments on evaporation from freely drained soils are described. Under isothermal conditions characteristic *winter* field behaviour is obtained, even when the air drying power is greater than its normal English midsummer value. Characteristic *summer* field behaviour is obtained when the rapid drying of a thin surface layer is achieved, either by using an extremely high air temperature under 'isothermal' conditions, or by raising the surface temperature by means of radiation—the normal method in nature. The effect of a high salt concentration in the soil water is shown to lead to greater evaporation losses and to a tendency for the salt to concentrate in the more salty patches.

It is suggested that mulching will only be beneficial during the isothermal part of the year, i.e. when soil surface and air temperature are approximately equal, and that it will have little effect on water conservation where the soil will be self mulched by the action of summer sunshine. The cause of this self-mulching action is briefly considered in the light of our limited knowledge of soil water dynamics; it appears to

depend on the existence of a dual mechanism of water movement in soils—as liquid and as vapour—the rates of movement being very different functions of moisture content and moisture gradient.

The author wishes to express his thanks to Dr R. K. Schofield for helpful discussions on the interpretation of these experiments.

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DISPERSION STUDIES ON GEZIRA SOIL

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(With Five Text-figures)

INTRODUCTION

It has been observed by several workers that the dispersibility of a soil in water depends on its moisture content. Puri & Keen (1925) studying this dependence, using English soils, found that the dispersibility at low moisture contents was substantially constant, but that (with Hoos soil) above 5% moisture the dispersibility increased linearly with the moisture content over a large range. With separated clay, on the other hand, they found that from the lowest moisture contents there was a linear increase in dispersibility with increasing moisture content. Many observations have been made at this station on the dispersibility of Gezira soil under varying field conditions. These results have been in general agreement with the observations of Puri and Keen. Briefly, moist samples from irrigated plots have shown a greater dispersibility than those from fallow plots when examined immediately after sampling. Most of this work has been done on top-foot samples, the field moisture contents seldom being less than 5%.

The present paper reports an investigation on Gezira soil in which it is shown that this heavy clay soil behaves differently, at low moisture contents, from the English soils studied at Rothamsted. It is shown that over a moisture range of nil to 5% the dispersibility of Gezira soil increases rapidly with *decreasing* moisture content. The different effects of oven drying on Gezira and English soils had earlier been noticed by Joseph & Snow (1929) in their studies of dispersion and mechanical analysis. They showed that using oven-dry Gezira soil did not hinder dispersion in their mechanical analysis procedure, as compared with the use of air-dry soil. With English soils this is not the case and the use of air-dry soil is preferred.

EXPERIMENTAL

Method I. Vigorous shaking

Samples of soil weighing 10 g. are added to 400 c.c. of water in a Wagner shaking bottle which is then rotated in a shaking machine. The contents, after shaking for a definite period, are poured into a wide bottle, and, with washings, are made up to 1 l. This is briefly agitated, and after standing for a period a 50 c.c. sample is withdrawn from around a depth of 10 cm. from the surface. This is evaporated to dryness on a water-bath, dried and weighed. Throughout the work 10 g. samples and a standard technique were employed, and the dry weight of suspended matter is taken directly as a measure of dispersibility. Preliminary experiments were done to enable suitable periods for shaking and sedimentation to be standardized.

Fig. 1 shows the relation between dry weight of suspended matter in 50 c.c. of suspension and the *period of shaking*, the period allowed for sedimentation being 2 hr. The figure shows the results for oven-dry and air-dry soil (the latter containing 6% of water). The original material in each case was long-stored Gezira surface soil, being part of a large sample stored in sealed tins, and referred to as Gezira standard soil.

It is immediately apparent that the oven-dry soil is more easily dispersible than the air-dry soil from which it was prepared. The slopes of the curves differ, and suggest that, after something like 6 hr. shaking, air-dry soil would suffer more dispersion than the oven-dry soil. Comparison with the corresponding curves given by Puri & Keen for the effect of time of shaking is difficult because their curves give no points for periods less than 5 hr. It may be observed, however, that their findings quoted above were based on experiments with 2 hr. shaking, as well as upon others with up to 24 hr. shaking. It is clear, therefore, that the different behaviour of Gezira soil is not attributable to differences in procedure.

Fig. 2 shows the variation of suspended matter with *time of sedimentation*, the samples having each been shaken for 30 min. Here the very different behaviour of oven-dry and air-dry samples is again noticeable.

From a consideration of Figs. 1 and 2, and for general convenience, it was decided to standardize 30 min. shaking and 2 hr. sedimentation, and to express the dispersibility directly as the weight of dry matter contained in the 50 c.c. sample, 10 g. of soil being used in all cases. It

was not considered necessary to work with 10 g. on oven-dry basis; this, of course, does not affect the general nature of the results.

A large sample of Gezira standard soil was soaked in water for 3 weeks, after which it was allowed to dry in the air, and, as this process continued, samples were taken from time to time, and were passed through a 10-mesh sieve. With the drier and more friable samples, some of the finer

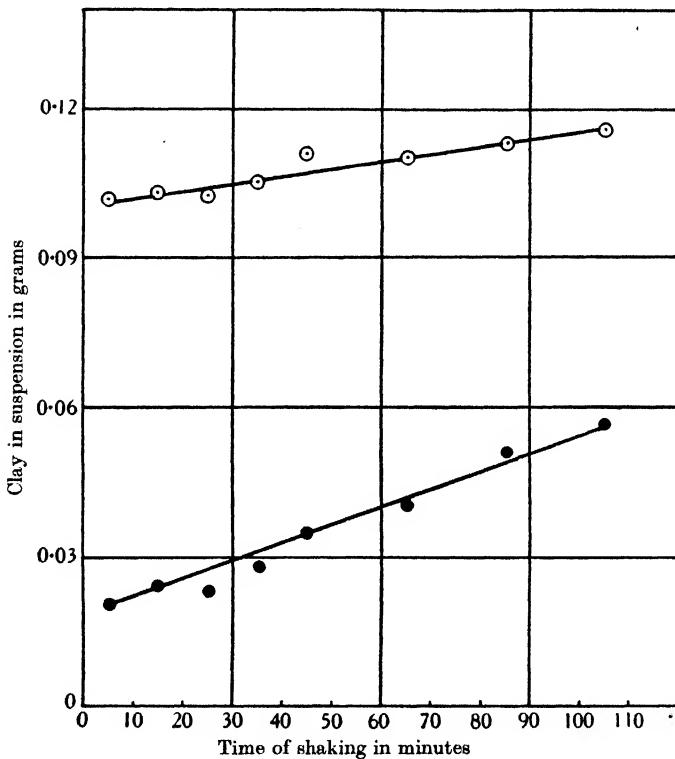


Fig. 1. Suspended matter against time of shaking.
 ○ Oven-dry soil. ● Air-dry soil. 2 hours' sedimentation.

soil was removed by the use of a $\frac{1}{2}$ mm. sieve, and the remaining coarser material was used in the experiments. This was done because it has been observed that the dispersibility of this soil depends to an appreciable extent on its particle size. Fine Gezira soil is noticeably less easily dispersible than a coarser sample, and steps were therefore taken to limit to some extent the range of particle sizes used in these dispersion experiments. After reaching the air-dry state further samples with decreasing moisture contents down to 'oven-dry' were prepared by

progressive drying in an oven at 105° C. The moisture contents of the samples were determined, and 10 g. of each were subjected to the dispersion treatment described above. With the equipment available it has not been possible to reproduce a given speed of shaking: a speed of approximately 50 rev./min. has been used throughout this work. The variation

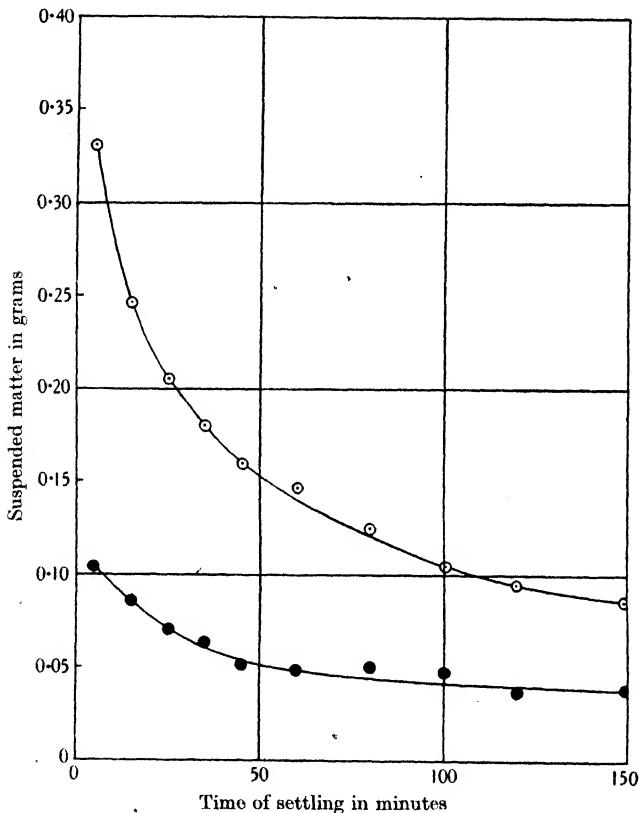


Fig. 2. Suspended matter against time of settling.
 ○ Oven-dry soil. ● Air-dry soil 30 minutes' shaking.

in speed of shaking has probably been confined to 5 rev./min. either way. A series of samples is of course shaken together.

Fig. 3 shows the results of this investigation.

The same procedure was followed with a moist sample taken from the field. This was from an irrigated plot, which for 4 months had had approximately fortnightly waterings. This sample gave a curve similar in form to that in Fig. 3. This curve differed, however, from Fig. 3 in

having the minimum dispersibility at 5 % instead of at 7 % moisture as in Fig. 3. Some difference in the dispersibility moisture curves is not surprising with two samples differing so widely in their history. The first experiment was done on soil which had been stored, air dry, for several years, and a 3 weeks' soaking would not be expected to return it to the state of a natural field sample. The study of this influence of previous treatment is excluded from this account.

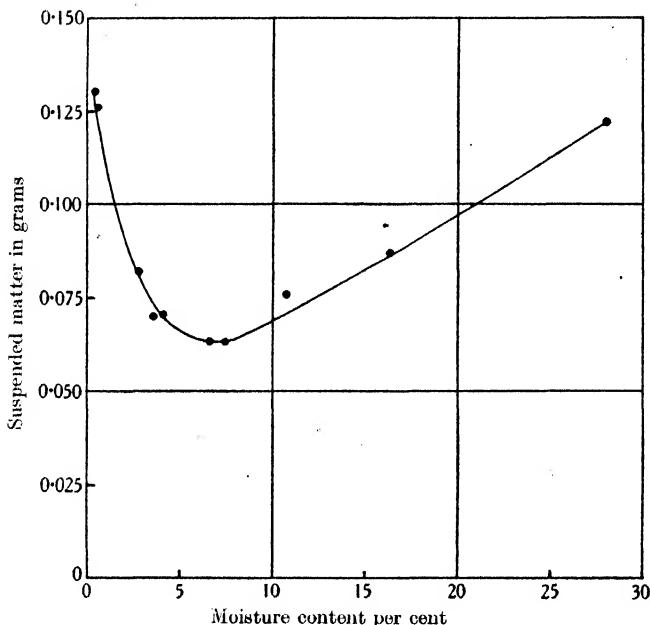


Fig. 3. Suspended matter against moisture content.

Method II. Dispersion without shaking

The two experiments reported above describe the dispersion brought about by vigorous shaking. From an agricultural point of view it is of greater interest to know how a soil behaves with water under more static conditions.

Fig. 1 shows that the curve connecting the dispersion with the time of shaking does not pass through the origin, that is, a certain amount of dispersion occurs immediately the soil enters the water. Puri & Keen also commented upon this; they found that the amount dispersed spontaneously in this way was practically zero for their air-dry samples and increased with initial moisture content.

In this present work the results of a more direct investigation of this spontaneous dispersion are reported. It was found that when powdered Gezira soil is sprinkled into water, a certain limited amount of dispersion at once takes place. The extent of this dispersion under a minimum of mechanical action can be easily estimated, and should be of interest in considering the behaviour of soil in the field, where dispersion takes place chiefly spontaneously and under the action of rain.

The method adopted is to sprinkle 10 g. of the powdered sample through 100 c.c. of water contained in a Nessler cylinder. Twenty hours later the top 50 c.c. are withdrawn by pipette, evaporated to dryness on a water-bath and weighed. The soil powder used should not be too fine. The usual size in this work was that passing a $\frac{1}{2}$ mm. sieve, and, with Gezira standard soil, consisted mostly of particles with nearly $\frac{1}{2}$ mm. diameter, with little very fine material. The aim is to get particles of such a size that they fall independently, and not, as very small particles do, in conglomerates. The withdrawal of a large sample by pipette inevitably introduces errors, but these with a standardized technique have not proved serious.

Fig. 4 illustrates how the weight of suspended matter varies with *period of sedimentation*. This curve was obtained using an oven-dry sample. An air-dry sample gave about ten times smaller weights of dispersed matter, and the scale of Fig. 4 prevents the effective depiction of the values. It is, however, established that oven-dry Gezira soil is considerably more dispersible than air-dry soil water, whether shaking is reduced to a minimum or is prolonged.

This variation of dispersibility with moisture content under conditions of minimum shaking was investigated by the above method over a moisture range of zero to 9% moisture, using as before Gezira standard soil.

Fig. 5 shows the dispersibilities as measured by the dry weight of suspended matter in the top 50 c.c. of the suspension. The curve shows to what a large extent the dispersibility varies between the air-dry and oven-dry states. It also appears that there is little further decrease in dispersibility beyond 9% moisture content. This has been separately verified as far as 11% moisture. Somewhat beyond this point the soil particles begin to cake together, and do not fall independently through the water, so that the results are not comparable with those obtained with drier samples.

Fig. 5 gives the results obtained with a series of samples which were prepared by progressive drying of moist samples. The same type of curve is obtained with samples prepared by allowing oven-dry soil to take up

varying amounts of water from a moist atmosphere. That is to say, within the moisture range dealt with, the effect of a short period of drying on dispersion is rapidly reversible. Thus, an oven-dry sample which gave 0.114 g. suspended matter, gave, after standing 50 min. exposed to the atmosphere, 0.026 g. suspended matter. It is important

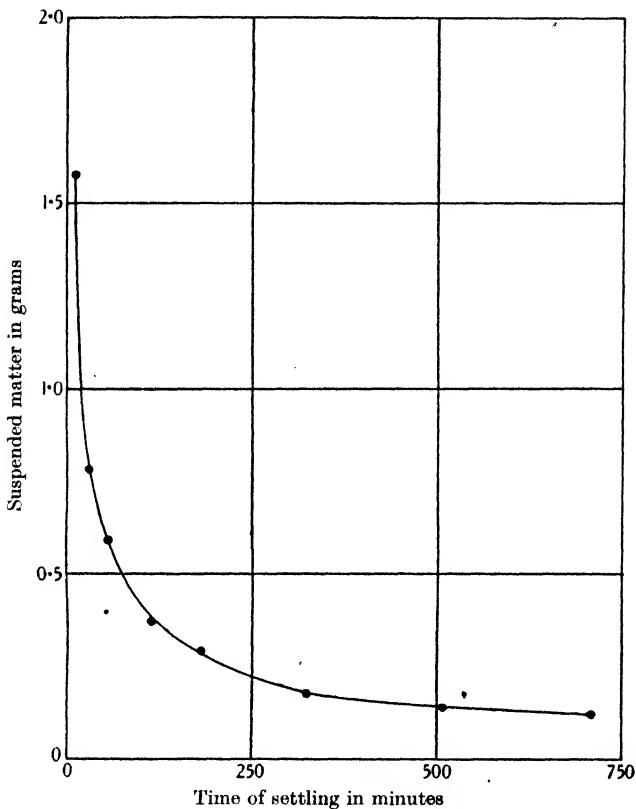


Fig. 4. Suspended matter against time of settling.

to note that long-period fallowing induces a change which is not quickly reversible, an effect which must be sharply distinguished from this rapidly reversible change.

Comparison of Figs. 3 and 5 shows that there are notable differences in the behaviour of the soil under the two methods of dispersion. The results of the second method do not show any increase of the dispersibility with increasing moisture content beyond the air-dry state, and do not

agree with Puri & Keen's conclusion that the spontaneous dispersion increases with the moisture content. The two methods of dispersion also differ in the relative change in dispersibility between the oven-dry and air-dry states. The ratio of dispersion with oven-dry soil to that with 5% moisture is about 2 : 1 with shaking and 6 : 1 without shaking.

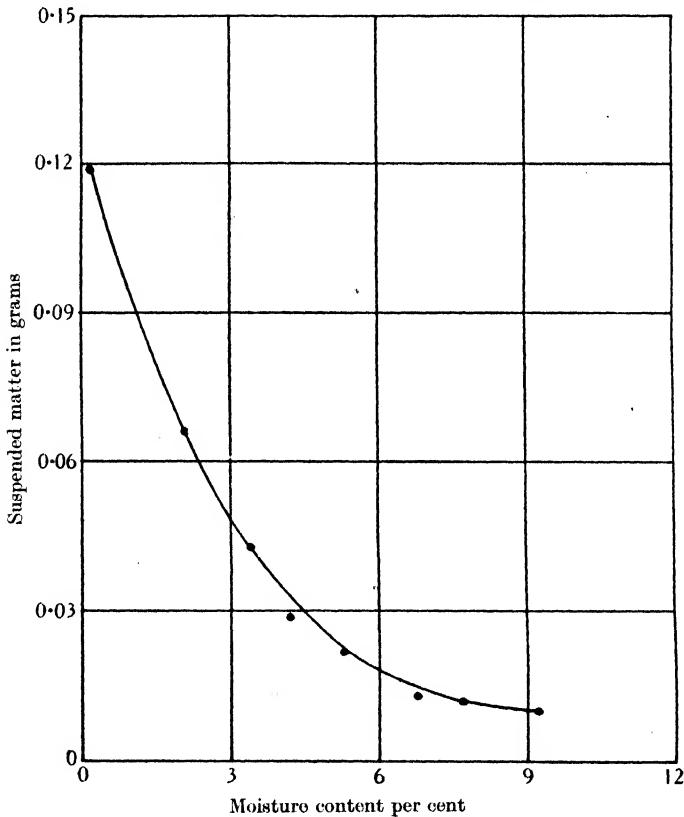


Fig. 5. Suspended matter against moisture content.

An idea of the actual extent of dispersion which takes place with and without shaking can be obtained from a scrutiny of Figs. 2 and 4, considering the values for oven-dry soil. Comparison is affected by the considerable difference between the sedimentation and sampling methods, and by the fact that at moisture contents close to oven dryness the dispersibility varies rapidly with the moisture content.

Considering the suspended matter after 100 min. sedimentation: these quantities are 0.105 g. with shaking and 0.40 g. without shaking,

each quantity being that in 50 c.c. of suspension. In the first case the 50 c.c. sample is one-twentieth of the total volume, in the second case it is one-half of it. Disregarding the variation in the amount of dispersed matter with depth from the surface, the total amount of suspended matter would be 2.1 and 0.8 g. respectively. It is evident that with easily dispersible oven-dry Gezira soil, spontaneous dispersion is responsible for an important part of the total dispersion achieved by shaking.

GENERAL DISCUSSION

It is well known that long-term fallow at the correct season brings about a marked improvement in physical properties of the Gezira soil. One of the ways which has been used here to follow this improvement in physical condition during fallowing has been the measurement of dispersion with shaking by a method similar in principle to that described in the present paper. The results over several seasons have shown conclusively that the surface foot of soil becomes less easily dispersible as fallowing goes on, and that irrigation only gradually increases the dispersibility. These results were obtained by dispersion of laboratory air-dried field samples at relatively similar moisture contents, and this effect must be carefully distinguished from that now under discussion, viz. that the dispersibility for any one soil varies according to the moisture content at the time of treatment with water.

At the end of the rains, the soil dries rapidly. A surface layer, about 0.5 cm. thick, becomes covered with a multitude of small superficial cracks at right angles to the surface. At the same time cracks develop parallel to and just below the surface, leading to the formation of a readily detachable surface crust divided into separate units. These cracks are distinct from the major vertical cracks which are a feature of the Gezira soil as drying out proceeds deeper.

On bare land which has not been cultivated there is no doubt as to what constitutes the surface crust. There is an obvious plane of weakness between it and the subsurface soil, and it is easily removable normally to the surface. It would seem likely that this crust formation is a legacy from the surface structure formed on bare surfaces of this soil during the rainy season. This surface crust acts like a mulch, since it reduces evaporation from the soil immediately below it. Between early morning and midday this crust shows large changes in moisture content; the subsurface soil immediately below the crust shows no such large variations. The crust evidently serves as a blanket, restricting interchange of

moisture with the atmosphere. The following figures illustrate this effect:

Percentage moisture content of samples

	8 a.m.						Average
Surface crust	6.2	5.9	6.0	5.1	5.3	7.5	6.0
Subsurface soil	6.4	5.9	7.1	4.4	7.5	6.3	6.3
1.30 p.m.							
Surface crust	3.0	3.5	3.0	3.2	2.5	2.9	3.0
Subsurface soil	7.0	7.0	5.5	4.9	6.0	6.2	6.1

It is clear that the dispersibility of this surface crust will vary during the day according to the moisture content when water is added; and considerable importance attaches to the dispersibility of surface soil as a controlling factor in the penetration of water.

It is probable that the maintenance of permeability is of prime importance to Gezira agriculture. The soil is unusually impermeable to water, and normal irrigations do not greatly affect the moisture content below the depth of about 3 ft. (Greene, 1928b). Cotton, the major crop of this region, has a root system which may extend to a depth of 5 ft. (Clouston, 1937), and so conservation and maintenance of moisture at the lower levels is of great importance. Before the sowing of each cotton crop in the Gezira the soil is not cropped for 2 years, and during this period it is exposed to two rainy seasons. It is these which offer a means of replenishing the subsoil moisture, and it is therefore important to consider any factor which may reduce or modify this replenishment. In this connexion the increased dispersibility of Gezira soil at the lowest moisture contents may have repercussions in the field of agricultural practice.

Duley & Kelly (1939), in Nebraska, sprinkled water at a known rate on to small field plots, and by measuring the run-off they were able to obtain the rates of intake. They showed that where the surface was protected by growing crops or debris, a greater rate of intake was obtained than on bare soil. They concluded that this was due to the crops and debris preventing the formation of a relatively impermeable surface layer by the impact of the artificial rain. Clouston (1938), at this station, has shown that surface puddling causes an important decrease in intake of water.

As far as the intake of rain water through the surface crust is concerned, it is doubtful whether such a relatively impermeable surface layer is of first importance in the Gezira until the major vertical cracks, already mentioned, are filled. During the rains large quantities of water

go down these large cracks, and at first have an unimpeded passage to the lower soil layers. With the water goes surface soil and mud formed by its dispersion, and this, with general swelling, closes the cracks. The filling of the cracks with surface soil leaves tongues of soil which are an easily recognizable feature of the soil in the Gezira, and have been described by Greene (1928a) from the point of view of soil structure and soil circulation. Clouston (1938), who has stressed the importance of the supply of moisture to the lower soil layers, has suggested that it is not only when the cracks are open that they provide an easy passage for water, but that, filled with surface soil, they continue for some time to provide an easy path.

The effectiveness of this mechanism for the supply of moisture to the subsoil depends on the permeability of the surface soil with which the cracks are filled. The importance of the instantaneous moisture content and the dispersibility of the surface crust of soil becomes clear, for the degree of dispersion of the surface soil which fills up the cracks must permanently affect their efficiency as water channels. The daily variation in the moisture content of the surface crust of soil, and the associated variation in dispersibility means that rain would effect less dispersion if the precipitation occurred during the night than it would during the day, for at night the soil moisture content is higher. Similarly, the dispersion effected by a rainstorm would vary from day to day with the saturation deficit and the moisture content of the surface crust.

The cracks fill up with dispersed surface soil during the earlier rains. The permeability of this soil depends on its moisture content at the time of dispersion. In actual fact the first rainstorms in May and June, months of intensive drying, occur mainly in the afternoon and early evening when the surface soil is in a vulnerable and dispersible state. After an early rainstorm during which partial filling of the cracks takes place, the surface soil may again become desiccated and easily dispersible, and well-spaced rainstorms may be cumulative in their effect on the efficiency of the cracks.

It was shown by Crowther (1926) that the yield of cotton sown in August is negatively correlated with the amount of early rains in May and June. Crowther & Crowther (1935) verified this up to 1931, but in later years the relation has broken down. No tenable explanation has been forthcoming for the earlier correlation. However, any consideration of seasonal differences would have to take into account variations which take place in the dispersibility of the intensively dried surface crust. The average monthly saturation deficit at midday in May and June is double

that of the average for July and August. The bulk of the season's rainfall is in July and August, and the heavier the rainfall in May and June the more seriously impaired is the efficiency of the cracks as water channels to the subsoil. The result is that relatively heavy rains in May and June adversely affect the subsequent passage of the later and heavier rains to the subsoil.

The importance of the dispersibility of the surface soil has been recognized by many workers on soil erosion. Middleton (1930) has measured the dispersion ratio of soils, a quantity related to the measure used for dispersibility in this paper, and to Puri & Keen's dispersion coefficient, and obtained by similar methods. He tentatively suggests that a division of soils into erodible and non-erodible classes could be made using the dispersion ratio. The influence of initial moisture content on dispersibility has also been investigated in soil erosion studies. The findings of different workers have differed: Diseker & Yoder (1936) found that a saturated surface lost much more soil than was lost by the same surface with only 10.8% of moisture, while Neal (1938) found under his conditions of erosion that there was most erosion at about 11% initial moisture. The relation between soil moisture and dispersibility discussed in this paper would have an importance in erosion work, if such a relation were found to hold for any of the soils subject to erosion in arid climates. The Gezira itself is a very level area, and erosion by water is not a problem.

SUMMARY

1. The degree of dispersion on shaking of the heavy clay Gezira soil has been studied in relation to its initial moisture content when it is added to the water. A minimum dispersibility was found at about 7% initial moisture content. Such behaviour is different from that found with certain English soils by Puri & Keen.
2. Spontaneous dispersion was studied using the same soil, and by this method it was found that the dispersion decreases with increasing moisture content over a range of zero to 9% moisture.
3. This relation between dispersion and water content is discussed in relation to possible effects in the field of practice. Attention is drawn to possible seasonal and daily differences in the effect of rainfall.

It is a pleasure to thank Mr O. W. Snow for his advice and encouragement during the course of this work and in the preparation of this paper.

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THE LIMITED NUMBERS OF NODULES PRODUCED ON LEGUMES BY DIFFERENT STRAINS OF *RHIZOBIUM*

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IN the field a legume crop usually obtains its nodules from a mixed population of nodule bacteria including a variety of strains doubtless varying in their effectiveness towards the host plant. It is therefore of practical importance to determine what are the factors that determine which strains will infect the plant and in what proportions. Nicol & Thornton (1941) found that an important factor controlling this was the competition that took place between bacteria of different strains *outside* the roots of the host plant. Where one was markedly dominant in competition, this became the determining factor controlling infection. But otherwise the relative infectivity of the strains determined the proportion of the total nodules contributed by each of them.

The numbers of nodules produced on a legume by a given strain of *Rhizobium* will clearly be conditioned by a number of factors, some arising in the root surroundings, others from the general physiology of the host plants, and others more specifically related to the strain of invading bacterium. It is with the latter more specific relationships that the present paper deals. Obvious differences exist between strains of *Rhizobium* not only as regards the mean size but also the mean number of nodules characteristically produced by them under conditions of host-plant cultivation rendered as carefully standardized as possible. These differences might be due to different rates of successful infection,¹ or to the establishment of a limiting equilibrium due to ability of a given strain to produce only a limited number of nodules on a given mass of the root system. If such a limiting equilibrium exists, the number of nodules per gram of root should attain a constant level characteristic of each strain. This number should remain constant on a growing root

¹ A large part of the root hair infections must fail to result in nodules (see McCoy, 1932). The term 'successful infection' is here used to designate infections that result in the formation of nodules.

system though the absolute number of nodules increases with the growth of the roots. On a root system that makes its growth over a short period, the further production of nodules should stop when the roots cease growing or when the specific limiting number of nodules per gram of root has been reached, whichever occurs last.

On this latter type of root system, if the limiting number of nodules per gram of root is quickly reached, the nodules on the young plant will comprise a large fraction of the number finally possible and hence will greatly reduce the formation of further nodules by the same or by a different strain. A number of authors have recorded such an inhibiting effect of early nodules. In particular, Dunham & Baldwin (1931) found that early nodules produced by one strain could entirely inhibit the formation of nodules by a different strain. Nicol & Thornton (1941), however, found that with peas and soy beans the inhibiting effect of the early nodules acted with equal intensity against the same as against a different strain. In this work which was designed to study competition between strains, the second strain was applied to sand already populated with the first, so that the results were complicated by competition between strains outside the plant. To measure the effect of the early nodules upon subsequent infection it is necessary to remove the bacteria, derived from the first inoculum, that remain in the root surroundings, and to transplant the roots into a medium populated only by the second inoculum. Experiments of this kind were made with the object of determining whether the nodule numbers per unit mass of roots attained a limiting equilibrium and how the establishment of this equilibrium affected subsequent infection by the same and by a different strain. These experiments were made with clover, whose root system continues its growth over a long period, and with soy beans, whose roots make most of their growth during early stages of culture.

EXPERIMENTS WITH RED CLOVER, 1939

In this experiment two strains of clover *Rhizobium* were used—the efficient strain 205 obtained from Wisconsin¹ and the inefficient Coryn strain, whose nodules were described by Chen & Thornton (1940). The rates of nodule appearance due to these strains on clover seedlings were found by Nicol & Thornton (1941) to be markedly different, while their

¹ The author's thanks are due to the staff of the Wisconsin Agricultural Experiment Station for supplying cultures of this strain and of the four strains of soy-bean nodule bacteria used in the second experiment, described below.

experiment in which clover was grown in sterilized sand showed that the absolute number of nodules produced in three months' growth by the two strains also differed characteristically.

In the present experiment, Montgomery red clover was sown in wide test-tubes on slopes of agar medium of the following composition:

K_2HPO_4	0.5 g.	NaCl	0.1 g.	$FeCl_3$	0.01 g.
KH_2PO_4	0.5 g.	$Ca_3(PO_4)_2$	2.0 g.	Agar	10 g.
$MgSO_4 \cdot 7H_2O$	0.2 g.	$FePO_4$	0.5 g.	Water	1 l.

The tubes of media were sterilized in the autoclave. Twenty replicates were left sterile, twenty supplied with strain 205 and twenty with the Coryn strain. The bacteria were mixed with the melted agar cooled to 42° C. before making the slopes. Two seeds, externally sterilized by immersion for 3 min. in absolute alcohol and for 3 min. in 0.2% $HgCl_2$ and washed with sterile water, were sown at the top of each slope. The seeds were sown on 13 February, and on 27 March the seedlings were removed from the tubes and their nodules counted. They were then replanted in small pots each containing 3 kg. of nitrogen-deficient sand, sterilized by blowing superheated steam through each pot for half an hour. 175 ml. of the following sterilized food solution was added to each pot:

K_2SO_4	0.9 g.	$FeCl_3$	0.02 g.
K_2HPO_4	0.5 g.	Boric acid	0.02 g.
$CaH_2(PO_4)_2 \cdot 4H_2O$	0.5 g.	$MnSO_4$	0.02 g.
$MgSO_4 \cdot 7H_2O$	0.5 g.	Tap water	990 ml.
NaCl	0.5 g.	Lucerne root extract	10 ml.

Twenty replicate pots were supplied with a heavy inoculum of strain 205 and twenty with one of the Coryn strain, the bacteria being mixed with the food solution before addition. One seedling bearing strain 205 nodules, one bearing Coryn nodules and two plants without nodules were planted in each pot, each plant being separately labelled. On 10 July the roots were washed, the nodules on each plant were counted and the dry weights of individual root taken. The results are shown in Table 1.¹ The experiment was so designed as to test whether any of the following factors had any effect upon the final nodule numbers per gram of root:

¹ The nodules per gram of root were separately calculated for each plant and the means of the figures so obtained are those shown in the table. They differ from those derivable from the mean nodule numbers (column 5) and the mean root weights (column 6). The same process was followed for the corresponding figures in Table 3.

(1) time at which the bacteria were first applied; (2) size of the root system as modified by the efficient strain applied at seeding time; (3) a possible inhibiting action of the early nodules against the same or a different strain.

Table 1. *Effect of strain of Rhizobium on nodule numbers in red clover*

Set	Strain applied		Mean nodule numbers per plant		Final root dry wt.		
	At sowing	After planting	When transplanted	At end	Means per plant	Final nodules per g. root	<i>n</i>
1	—	Coryn	—	357.2 ± 42.8	118	3402.4 ± 475.8	17
2	Coryn	Coryn	38.7 ± 4.4	385.5 ± 74.0	163	2770.9 ± 558.3	10
3	205	Coryn	7.3 ± 0.8	904.1 ± 181.4	381	2273.7 ± 360.9	14
4	—	205	—	182.1 ± 30.8	355	544.8 ± 56.8	15
5	Coryn	205	50.0 ± 1.4	169.3 ± 38.4	239	694.5 ± 101.3	10
6	205	205	9.1 ± 1.5	331.1 ± 50.7	573	652.6 ± 121.3	14

At the time of planting out, seedlings that had grown for 6 weeks on agar already showed differences in nodule numbers characteristic of the strain supplied at seeding time (column 4). When removed from the agar the seedling root system showed no differences in size according to the culture supplied—the efficient nodules not having had time to produce increased growth.

After transplanting into the pots some plants died. The numbers surviving can be deduced from the degrees of freedom, *n*, shown in the last column. These made considerable growth before harvest with a large increase in nodule numbers. The final root weights shown in column 6 were much increased where 205 nodules, effective in nitrogen fixation, had developed on the seedling while growing in agar. This appears in comparing set 1 with 3 and set 4 with 6.

The absolute number of nodules at the end of the experiment (column 5) were not significantly affected by the presence of Coryn nodules on the seedlings but the presence of 205 nodules at the time of transplanting greatly increased subsequent nodule formation by either strain. This effect was in fact due to the enlargement of the root system resulting from nitrogen fixation by the early-formed efficient strain 205 nodules.

The nodules per gram of root (column 7) show no significant differences between sets receiving different treatments in their early growth but later grown in pots supplied with the same strain. Thus the time at which the bacteria were first supplied to the roots was without final effect on the nodules per gram of root. Nor were there very large

differences in size of the root system produced by the early formed 205 nodules. There were, on the other hand, large differences in the mean number nodules per gram of root according to the strain in the sand which was in contact with the root system during the period when it made most of its growth. This mean number reached a definite limit characteristic of the strain present in the sand. This limit was apparently attained quite early in the plant's growth. The mean figure for plants grown in pots containing Coryn bacteria was 2816, and that for plants in pots containing strain 205, only 631 nodules per gram of root. These figures are in the ratio of 4.6 : 1. This ratio can be compared with that between the actual nodule numbers developed by the two strains in agar (column 4), because during this early period the size of the root systems was similar in all sets. The Coryn strain developed a mean of 44.4 nodules per seedling, and strain 205 a mean of 8.2, at the time of transplanting. These figures are in the ratio of 5.4 : 1. So that the two strains produced nodules whose numbers per unit of the root system were in approximately the same ratio both on seedling roots grown in agar and subsequently on plants grown in pots of sand. Thus the limit of infection for a root system of given size is characteristic of each strain and is quickly reached. But the absolute nodule numbers of nodules increased *pari passu* within the growth of the root system, which in clover continues over a long period. This explains why the presence of nodules on the seedling did not stop further nodule formation, which took place on a growing root system, and why the final number of nodules per gram of root was that characteristic of the second applied strain, which was in contact with the root system while this was making most of its growth.

The following experiment, similar in general design to the first, was made with soy beans, whose root system makes most of its growth when the plant is quite young. It was designed to determine what specific limits of nodule numbers per gram of root were possessed by four strains of soy bean *Rhizobium* and to test the influence of the early nodules upon later infection by the same and by different strains.

EXPERIMENT WITH SOY BEANS, 1939

Soy beans were grown in glazed earthenware pots each containing 12 kg. of sand and 1 l. of food solution similar in composition to that used in the first experiment. Five seeds externally sterilized were sown in

each pot on 21 June. Eight replicate pots were left uninoculated and eight each were supplied with each of the following strains of *Rhizobium*:

Wisconsin 501	Effective
„ 505	
„ 502	Ineffective
„ 507	

The plants were grown for 9 weeks and their roots were thoroughly washed and the nodules counted. They were then replanted in the pots in such a way that each pot whose sand contained an effective strain (501 or 505) received one plant bearing nodules produced by the same strain, one plant bearing nodules produced by each of the ineffective strains and one uninoculated plant. Similarly each pot whose sand contained an ineffective strain (502 or 507) received one plant bearing nodules produced by the same strain, one plant bearing nodules produced by each of the effective strains and one plant without nodules. Each plant was separately labelled. The scheme of transplanting is shown in Table 2. After a further 14 weeks' growth the nodules were recounted

Table 2. *Soy-bean experiment, scheme of transplanting*

Plants with nodules, when transplanted, of strain	Transplanted into pots whose sand contained strains			
	501 Set	502 Set	505 Set	507 Set
501	1	2	—	3
502	4	5	6	—
505	—	7	8	9
507	10	—	11	12
No nodules	13	14	15	16

and dry weights of the roots were taken. The results are shown in Table 3. The plants which bore nodules produced by strains 501, 502 or 505 before transplanting did not show any significant increase in nodule numbers after transplanting (sets 1-9, columns 4 and 5). Thus the limit of nodule numbers attainable on the root systems in these sets had been reached within the first 9 weeks' growth. The number of nodules per gram of root was specific to the strain of *Rhizobia* (column 7). The mean number of nodules per gram of root in sets 4, 5 and 6 which bore nodules produced by strain 502, was 284.7, a figure significantly higher than the mean numbers, 191.7 and 163.4 of the sets whose nodules were produced by strains 501 and 505 respectively (sets 1-3 and 7-9).

The plants without nodules at the time of transplanting made considerably greater root growth during the second growth period, probably

Table 3. *Effect of strain of Rhizobium on nodule numbers in soy beans*

Set	Strain applied		Mean nodule numbers per plant		Final root dry wt.		Final nodules per g. root	n
	At sowing time	After transplanting	When transplanted	At end	mg.	Mean per plant		
1	501	501	17.7	16.1 ± 2.7	94	206.3 ± 30.2	9	
2	501	502	17.2	18.5 ± 1.5	162	149.8 ± 38.6	4	
3	501	507	22.4	22.6 ± 3.4	144	219.1 ± 68.4	6	
		Mean				191.7 ± 25.9	21	
4	502	501	29.1	29.4 ± 4.8	123	269.1 ± 44.7	8	
5	502	502	35.9	37.8 ± 4.4	158	284.3 ± 38.1	7	
6	502	505	36.3	36.5 ± 6.1	131	300.8 ± 46.9	9	
		Mean				284.7 ± 24.6	26	
7	505	502	18.9	21.0 ± 1.7	170	134.4 ± 20.9	6	
8	505	505	22.5	19.6 ± 2.0	114	184.1 ± 29.9	7	
9	505	507	18.4	19.3 ± 2.0	185	171.7 ± 50.6	6	
		Mean				163.4 ± 19.6	21	
10	507	501	28.0	34.6 ± 4.7	77	451.0 ± 56.5	6	
11	507	505	29.6	44.6 ± 8.9	101	446.0 ± 49.4	7	
12	507	507	32.1	56.6 ± 10.1	141	436.2 ± 53.5	8	
		Mean				444.4 ± 29.4	23	
13	—	501	—	30.9 ± 7.4	179	211.4 ± 26.0	7	
14	—	502	—	70.3 ± 13.4	303	233.0 ± 39.7	8	
15	—	505	—	43.0 ± 8.8	244	186.4 ± 27.4	7	
16	—	507	—	57.0 ± 13.4	253	225.3 ± 43.6	7	

because they were smaller at the time of transplanting and suffered less check. These plants in sets 13, 14 and 15, planted in sand containing bacteria of strains 501, 502 and 505 respectively, developed nodules whose numbers per gram of root did not differ significantly from those on plants that had received the corresponding strain at the time of sowing (compare sets 1 and 13, 5 and 14, 8 and 15). Thus the specific limit of nodules per unit mass of root system was attained regardless of the total mass of the root system, which varied widely, or the time at which the infection took place. This latter point shows that the number of nodules is determined by the size of the root system and not vice versa, since most of the root growth in sets 13, 14 and 15 took place before the plants had developed any nodules. Strain 507 has a much higher level of nodule numbers than the other three strains. The mean final nodule numbers per gram of root for sets 10, 11 and 12 was 444.4, a figure significantly higher than that for any other set or group of sets. This high figure was not reached during the period of 14 weeks' growth in set 16 which first received the bacteria at the time of transplanting. Nor was the full number of nodules reached during the first 9 weeks of seedling growth in sets 10, 11 and 12, which developed more nodules after transplanting. In sets 10 and 11 these additional nodules were in fact

produced by the strains 501 and 505 respectively as was shown by examining the nodules.¹ These later-formed nodules were comparatively few and the number of nodules per gram of root finally reached was that characteristic of strain 507. The figures for sets 10 and 11, 451 and 446, do not differ significantly from that of 436.2 for set 12 which received strain 507 both at sowing time and after transplanting.

DISCUSSION

In the experiments described above the number of nodules n divided by the dry weight of the roots m was found to reach a limiting figure that was constant and specific for each strain of *Rhizobium*,

$$n = mk.$$

If a plant's roots are exposed in succession to pure cultures of two strains of *Rhizobium* having the limiting constants k_1 and k_2 and if each strain is allowed time to reach its limit, the number of nodules n_1 produced by the first strain will be $m_1 k_1$ where m_1 is the mass of the roots developed while in contact with this strain. On the simplest supposition, the number n_2 produced by the second strain will be $m_2 k_2$ where m_2 is the additional mass of roots developed in contact with it. The total nodules developed by the two strains will therefore be

$$n_1 + n_2 = m_1 k_1 + m_2 k_2.$$

In the experiment with clover, nearly all the root growth took place after transplanting so that m_2 was very large relatively to m_1 . Consequently the number of nodules was determined by k_2 and was that characteristic of the second-applied strain. In the soy bean experiment, all or nearly all the root growth took place in the presence of the first-applied strain, whose specific constant, k_1 , determined the limit of nodule numbers reached.

It would be interesting to investigate the condition where the host is removed into the presence of the second strain before the first strain had reached its limit of infection for a given mass of roots, and to discover to what extent the first strain can then impose its specific limit on further nodule formation in these same roots by the second strain. Thus if the number of nodules produced by the first strain, $n_1 = m_1 k_1 - x$, would the number x , produced by the second strain, be determined by

¹ Nodules produced by the effective strains 501 and 505 have soft reddish centres easily distinguishable in hand sections from the hard whitish centres of the ineffective nodules produced by strain 507 (see Nicol & Thornton, 1941).

the constant k_1 or by a constant k_2 specific to the second strain? The answer to this question might throw light on the mechanism of nodule limitation. The evidence from sets 10 and 11 in the soy bean experiment suggests the continued operation of the constant k_1 specific to the first strain, but this evidence is insufficient to form any basis for discussion.

SUMMARY AND ABSTRACT

Pot experiments were made with red clover and with soy beans to determine how far the number of nodules developed was a specific character of the strain of *Rhizobium* supplied.

The number of nodules per gram of root was found to reach a limit specific to each strain. This limiting equilibrium was attained regardless of the size of the root system or the age of plant at which the culture was first supplied, provided enough time were allowed for the limit to be reached.

When two different strains were applied to the root surroundings in succession, the final number of nodules was determined by the limit specific to the strain in contact with the roots while these were making most of their growth. In clover this was the second and in soy beans the first applied strain.

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NOTE CONCERNING AUTHORSHIP

The work described in this paper was carried out by Dr Chen shortly before his departure for Central China. Some difficulty in communication due to wars has made it necessary for the undernamed to write the paper from Dr Chen's notes and data without his having the opportunity to see it before publication. The writer thinks he has drawn conclusions from the data in agreement with Dr Chen's opinions, but he accepts full responsibility for these conclusions and for the actual writing, although credit for the work is due solely to Dr Chen.

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THE EFFECT OF BECKMANN'S TREATMENT BY SODIUM HYDROXIDE ON THE DIGESTIBILITY AND FEEDING VALUE OF BARLEY STRAW FOR HORSES

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UNDER conditions created by the war, renewed attention has been given to the preparation of straw whereby its utmost value as a food may be realized. It is now generally accepted that of all the methods devised Beckmann's process, by which the straw is steeped for at least 3 hr. in about 8 times its volume of a 1.25-1.5% solution of caustic soda, and is subject to thorough washing before feeding, is the most useful, where expensive plant or labour is not permissible.

The application of the method to conditions in this country has been fairly fully tried out, and feeding trials have been made on ruminants. On the other hand, there appears to have been no attempt made to confirm or refute the high value ascribed to it as a horse food in Germany during the last war, although if the claim is substantiated it might be of importance to our horse owners now.

Ellenberger & Waentig (1919) found that when rye straw prepared by Beckmann's process was fed to horses along with crushed oats the digestibility coefficients of the nitrogen-free extractives and the fibre of the mixture were raised from 25.30 to 76.64 and from 27.08 to 83.37 respectively, but that in the preparation of the straw 26.9% of the total dry matter was lost.

Fingerling (1917), using straw pulp prepared with caustic soda and the prolonged action of steam, found that the digestibility for horses of the total dry matter was 74%, that of the nitrogen-free extractives 65%, and of the fibre 82%. The pulp, he says, was palatable and readily eaten by horses.

In a digestibility trial carried out by Weiser & Zaitschek (1920) the digestibility coefficients of wheat straw when fed with oats to horses were found to be 35.3 for organic matter, 36.6 for nitrogen-free extracts and 33.4 for fibre: after the straw had been subjected to the action of 1.5% NaOH and steamed for 4 hr. under a pressure of 4.5-5 atm. the

respective figures were 47.9, 40.6 and 55.5. The starch value had been increased from 11.34 to 32.73.

V. d. Heide *et al.* (1915) asserted that cereal straw prepared by boiling in caustic soda to which 20% of molasses had been added was equal to two and a half times its weight of hay and all but a tenth of its weight of oats as a source of energy.

EXPERIMENTAL DETAILS

Material used and method of sampling

In testing the usefulness of Beckmann's process for the treatment of straw for horse food it was decided to use barley straw rather than that of oats or wheat because throughout the Empire, though not in the British Isles, that is the straw most commonly fed. It was considered advisable that the straw should be a supplement to a basal ration of such material as is commonly fed to horses, provided that the basal diet provided sufficient protein and was not too bulky. A ration consisting of 454 g. crushed oats, 227 g. flaked maize, and 227 g. medium bran with 9 gm. of NaCl per day was selected as likely to fulfil these requirements. The ingredients were bought in quantities judged sufficient for the whole experiment, but the work extended and more of the foods had to be obtained. The method employed for sampling was that described by Stewart (1929-30). The usual methods of analysis were followed.

The percentage composition of the ingredients of the first consignment of the basal ration, used up to 10 August, representing the average of four samples, was:

	Oats	Maize	Bran
Crude protein	11.95	9.95	15.76
Ether extract	3.91	3.30	3.95
Nitrogen-free extractives	59.48	70.58	53.26
Crude fibre	9.06	1.52	8.62
Total ash	2.41	0.88	4.95
Dry matter	86.81	86.26	86.54

The second consignment, again the average of four samples, was of the following composition:

	Oats	Maize	Bran
Crude protein	11.81	9.64	14.01
Ether extract	4.66	5.74	3.18
Nitrogen-free extractives	59.02	68.27	56.23
Crude fibre	9.89	2.05	8.85
Total ash	2.76	1.60	5.45
Dry matter	88.14	87.27	87.72

Two consignments of chopped barley straw were used. The straw was delivered in sacks and because in 'bulking' or 'quartering' and, in

fact, at every handling, the finer material tended to separate from the coarser and the heavy dust from the lighter, and as the sacks had been filled direct from the chaffer, it was thought that a fairer sample was obtained by taking random samples under the conditions laid down in the Fertilisers and Feeding Stuffs Regulations, 1932, than by any other way, and that was the method used.

The treated straw was sampled thus: On any day, selected at random, 3 lb. of straw were treated in exactly the same way as those lots which were to be used as food within the next 24 hr. At the time when it would have been fed if it had been intended for that purpose, it was dried on a hot plate in air, weighed, milled and a suitable portion of the powdered material taken for analysis.

The straw was a year old and of inferior quality as judged by sight and smell. Analysis showed the composition of its dry matter to be:

	Consignment 1		Consignment 2	
	Untreated		Untreated	Treated
Crude protein	3.37		2.80	1.95
Ether extract	0.84		1.00	0.85
Nitrogen-free extractives	43.32		44.24	38.38
Crude fibre	47.18		45.55	52.52
Total ash	5.29		6.40	6.31

The treatment thus resulted in a loss of crude protein, ether extract, and nitrogen-free extractives, and a relative increase in crude fibre. During the process an average of 14.47% of the original dry matter was lost.

The first consignment was used up to 12 May.

The straw for treatment was weighed out each evening, placed in ten times its weight of a 1.25% solution of caustic soda, and left there to soak for 12 hr. It was then washed until nearly all feeling of soapiness had disappeared and drained for 3 hr. before use.

The basal ration too was weighed out each day and was fed, the first half in the morning and the second half in the evening, mixed with somewhat less of the straw than what it was judged the pony would eat throughout the intervening period. After the mixture had been consumed, straw alone was offered until the time when more mixture was due.

Animals and apparatus used and conditions of management

The animals selected for the investigation were:

'C': a 5-year-old male Shetland pony brought in from grass some 6 months before the experiment began and kept in a loose box along with other ponies on a bare maintenance diet under conditions in which

infestation with entozoa could hardly be avoided. The food consisted almost entirely of Italian rye grass hay. The bodily condition of the animal was very poor, its weight being 127 kg.

'D': a 3-year-old male Shetland pony brought in from comparatively rich grazing and put directly on to the feeding platform. His bodily condition was very good, his weight was 165 kg. and he appeared to be in perfect health. While on the feeding platform, however, he suffered twice from serious attacks of colic; the first occurred a week after the experimental feeding began, and it necessitated his removal for 7 days; the second commenced on the day it was intended to start feeding the treated straw and on this occasion he had to be taken off the platform for a fortnight. When he was again fed on the untreated straw throughout period 3 a careful observer could see signs indicative of slight abdominal uneasiness.

'E': a pony about twelve years old and of uncertain breeding. For a year previous to the experiment it had been stabled, well fed on rye grass hay, oats, and other cereals, and well cared for. During the year it had been twice treated for intestinal worms as a prophylactic measure. Its condition was very good and it weighed 155 kg.

The extent to which each horse harboured entozoa was indicated by the number of the eggs of parasites found in the dung before and at frequent intervals throughout the period of investigation. Judged in that way, horse C was fairly heavily affected, horse D lightly so, and horse E was practically free. As under the experimental conditions fresh infection was well nigh impossible, no significant change in the count occurred.

All three animals were maintained in experimental conditions for one week previous to the beginning of the investigation. So as to facilitate the collection of faeces and urine, each horse was housed on a stout wooden platform erected 2 ft. above the cement floor of an ordinary horse stall. At a convenient point an aperture in the platform overhung the mouth of a 2-gallon glass jar placed below it. A bicycle tube, connected to the outlet of a light leather conical bag fitted over the prepuce of the horse ran through the aperture into the mouth of the jar in which it was suspended in position by a weight of lead piping fitted into its extremity. The base of the bag was moulded to shape and strengthened to rigidity by a piece of flexible steel wire which was sewn into it. A close enough proximity to the skin was assured by six light straps, three on either side, which, rising from the thigh, two over the flank and one over the perineum, were attached by buckle couplings

to a crupper. The bag itself was of sufficient size to contain all splashings but not so large as to hinder movement or handling. The design was an adaptation of the model used for bullocks at the Hannah Dairy Research Institute, Kirkhill, Ayr.

The faeces were caught in a bag made after the design of those commonly used for digestibility experiments with ruminants, but it was found very convenient to have it of such a length that while its base rested on a stand placed some inches from the ground, it allowed what freedom of movement was otherwise permitted the horse without constituting a dragging weight which would tend to inconvenience the animal and displace the gear.

A back strap held in position by a breast strap, roller and crupper was the ultimate support of both urine and faeces bags.

The platform was of a length which allowed one full step forward or backward and of a breadth which was just sufficient to allow the horse to lie down. No restricting device behind was required, for each horse soon learned the posterior limits of the platform and its height from the ground made them so shy of stepping over it that after one or two attempts they desisted.

The apparatus proved satisfactory, the animals quickly grew accustomed to it, and they were evidently comfortable in it, for they were eager to return to it after their exercise. No bedding was provided and horse D was the only one that was known to lie down.

Exercise was necessarily of a very restricted nature and consisted of a walk in an open yard for about a quarter of an hour each day. Grooming was carried out each morning.

In the early stages of the trial the amount of food offered was governed only by the amount the animals would eat, but later when each horse's capacity became known, the greatest amount which it was judged the animal would eat up within half an hour was given. At least such was the case as regards horses C and E, but it was found that horse D would gorge whenever it was given the opportunity and so the straw fed to it was curtailed to an amount which was empirically judged to be adequate.

The question of the palatability of the treated straw caused no misgiving, for it was eaten with zest by all three horses.

The faeces were collected twice daily, weighed, broken down and mixed by hand. A random sample was then taken and, after the water content had been ascertained, it was dried and an aliquot part of each day of an analytical period was mixed and the composite sample stored for analysis.

The faeces altered very little in consistency throughout the whole experiment, but they were of a lightish pasty colour during the time the treated straw was being fed.

The urine was collected each morning, its volume measured, and an aliquot part of each day of an analytical period was mixed as a composite sample with H_2SO_4 and stored for analysis.

The food residue was lifted every 24 hr. It was dried, weighed, milled, and an aliquot part treated in the same manner as that of the faeces. It was invariably found that food residue was composed entirely of the coarser parts of the straw.

The necessary current analysis was undertaken twice a week, that is, at 3 and 4 day intervals. The results are summarized in three periods, namely, a first period between 21 April and 4 June when untreated straw was fed, a second period between 5 June and 6 August when treated straw was used, and a third period between 7 August and 3 September when the untreated straw was reintroduced.

Digestibility coefficients of the diet

Horse C

	Crude protein	Ether extractive	Nitrogen-free extractives	Crude fibre	Organic matter
g. g. g. g. g.					
First period (44 days recorded)					
Net consumption	6905.35	2050.37	51829.05	32373.58	93158.35
Voided	2651.78	918.73	22984.82	20957.41	47512.74
Digested	4253.57	1131.64	28844.23	11416.17	25654.61
Digestibility coefficients	61.60%	55.20%	53.73%	35.26%	49.00%
Second period (58 days recorded)					
Net consumption	7736.51	2460.68	55298.74	36076.09	101572.02
Voided	2878.85	1169.11	16548.22	14278.95	34875.13
Digested	4857.66	1291.57	38750.52	21797.14	66696.89
Digestibility coefficients	62.78%	52.50%	70.39%	60.42%	65.66%
Third period (28 days recorded)					
Net consumption	3935.27	1466.08	30059.07	17016.83	52477.25
Voided	1438.20	586.33	12801.66	10677.61	25493.80
Digested	2497.07	879.75	17257.41	6339.22	26973.45
Digestibility coefficients	63.46%	60.10%	57.41%	37.25%	51.40%

Horse E

First period (44 days recorded)					
Net consumption	7593.59	2250.19	61254.37	42131.64	113236.42
Voided	3680.21	1175.45	29268.11	25246.41	59367.57
Digested	3913.38	1074.74	31986.26	16885.23	53868.85
Digestibility coefficients	51.53%	47.72%	52.22%	40.08%	47.57%
Second period (54 days recorded)					
Net consumption	7634.60	2484.98	60139.64	45442.88	115697.21
Voided	3535.66	1569.41	20339.36	17375.00	42811.39
Digested	4098.94	915.57	39800.28	28067.79	72885.82
Digestibility coefficients	53.69%	36.24%	66.18%	61.76%	63.00%

	Crude protein g.	Ether extractive g.	Nitrogen-free extractives g.	Crude fibre g.	Organic matter g.
Third period (28 days recorded)					
Net consumption	4128.58	1527.98	32886.45	19951.71	58494.98
Voided	1815.23	711.76	14781.90	12380.36	29689.34
Digested	2313.35	816.22	18104.55	7571.35	28805.64
Digestibility coefficients	56.03%	53.41%	55.05%	37.95%	49.24%

Horse D

	First period (37 days recorded)				
Net consumption	7003.94	2107.94	61228.68	45470.01	115807.38
Voided	3491.77	1288.75	31961.65	30898.83	67635.71
Digested	3512.17	819.19	29267.03	14571.18	48171.67
Digestibility coefficients	50.14%	38.86%	47.80%	32.04%	41.60%
	Second period (48 days recorded)				
Net consumption	7510.25	2516.44	67400.51	59515.55	136937.12
Voided	4631.41	1890.13	31093.13	34129.38	71735.99
Digested	2878.84	626.31	36307.38	25386.17	65201.13
Digestibility coefficients	38.33%	24.90%	53.87%	42.65%	47.61%
	Third period (28 days recorded)				
Net consumption	4575.14	1713.65	40206.78	27406.57	73893.55
Voided	2327.69	956.65	20706.52	18761.31	42743.07
Digested	2247.45	757.00	19500.26	8645.26	31150.48
Digestibility coefficients	49.13%	44.20%	48.50%	31.54%	42.16%

The digestibility coefficients over the whole period are:

	Crude protein %	Ether extractive %	Nitrogen- free extractives %	Crude fibre %	Organic matter %
Horse C					
Diet containing untreated straw	62.53	57.65	55.57	36.26	50.20
Diet containing treated straw	62.78	52.50	70.39	60.42	65.66
Horse E					
Diet containing untreated straw	53.78	50.57	53.64	39.02	48.41
Diet containing treated straw	53.69	36.84	66.18	61.76	63.00
Horse D					
Diet containing untreated straw	49.64	41.53	48.15	31.79	41.88
Diet containing treated straw	38.33	24.90	53.87	42.65	47.61

The depreciation in digestibility of the ether extracts caused by the treatment is quite pronounced, although that of the crude proteins appears to be little affected, but as both of them form such a small constituent of the treated straw their digestibility is of no practical significance.

The results shown by horses C and E are close enough to indicate that they may represent the average for normal horses. Those from D reflect the relatively inefficient manner in which that horse digested the diet—an inability which had already been expressed in clinical signs of abnormality.

The intervals in which the records of horse D were interrupted by illness (colic) were from 28 April to 4 May in the first period, and from 5 to 18 June in the second period. In the first instance there was acute illness for 1 day and in the second for 5 days. Time was given in both instances for the animal to recover fully before records were again taken, but the diet was not changed during that time, although the amount consumed was very much less than usual.

Digestibility coefficients and starch value of the straw

Calculated from Kellner's digestibility coefficients for oats and maize for the horse and those of dry bran for the ruminant, the digestibility coefficients for the straw in this experiment are:

	Crude protein %	Ether extractive %	Nitrogen-free extractives %	Crude fibre %	Organic matter %
Horse C					
Untreated straw	20.25	28.40	32.0	36.79	35.41
Treated straw	?*	?*	56.6	63.1	50.20
Horse E					
Untreated straw	2.86	10.67	34.45	39.68	37.79
Treated straw	?*	?*	51.68	64.31	56.39
Horse D					
Untreated straw	4.3	0.7	30.73	31.83	30.1
Treated straw	?*	?*	36.51	43.28	37.75

* Negative values were obtained, but the quantities involved are so small that for practical purposes they can be ignored.

Using Kellner's method and his correction figure of minus 0.58 lb. starch equivalent for each 1% of fibre in the chemical composition, the starch equivalent of 100 lb. of the straw, dry matter, is:

	Untreated	Treated
Horse C	5.26	24.00
Horse E	5.90	23.15
Horse D	1.34	6.28

Although the straw was chaffed the correcting figure of 0.58 is used rather than that of 0.29 in view of the fact that the difference between the theoretical and the actual feeding value of fibre for the horse has been shown to be 2.65 cal. (Zuntz & Hagemann, 1898; Zuntz & Lehmann, 1889).

The digestive inability of horse D is again reflected here, so in computing the value of the straw one may ignore the results obtained from that animal. The mean value as starch equivalent of 100 lb. of the straw, dry matter, is then 5.53 lb. for the untreated straw and 23.58 lb.

for the treated straw. Taking into account the 14.5% of dry matter lost in treatment, the starch equivalent value of the treated product from 100 lb. of the straw is 20.16 lb.

Again using Kellner's figures for composition and digestibility coefficients, poor quality meadow hay has a value for the horse of 17.88 lb. starch equivalent per cent. Thus the treated straw was of a somewhat higher value for energy production than poor quality meadow hay.

Nitrogen balance

In feeding a diet composed so largely of a bulky material which contains so little protein, the importance of maintaining a positive nitrogen balance was realized. For the first week or two in each case the balance was to a very slight degree negative, but after that a positive balance was maintained by each animal, or if it did veer to the negative side, the deficiency was of short duration and small enough to be of little account.

Period	No. of days recorded	Nitrogen consumed g.	Nitrogen voided (g.)			Total	Mean daily nitrogen balance g.
			In faeces	In urine			
Horse C							
21 Apr. to 4 June	41	6458.80	2497.49	3992.40	6489.89	— 0.78	
5 June to 6 Aug.	43	5725.35	2141.22	3370.38	5511.60	+ 4.97	
7 Aug. to 3 Sept.	21	2934.92	1063.93	1631.60	2695.53	+ 11.40	
Horse E							
21 Apr. to 4 June	41	7106.99	3501.88	3429.71	6931.39	+ 14.28	
5 June to 6 Aug.	52	6928.46	3246.49	3061.82	6308.31	+ 11.93	
7 Aug. to 3 Sept.	25	3515.28	1685.57	1500.00	3185.57	+ 13.19	
Horse D							
21 Apr. to 4 June	37	7103.94	3284.62	3920.70	7205.32	— 2.74	
5 June to 6 Aug.	37	4193.85	2517.95	1597.50	4115.45	+ 7.13	
7 Aug. to 3 Sept.	22	3602.36	1875.19	1610.60	3485.79	+ 5.30	

Amount of straw consumed and its bulk

Estimated as dry matter, the amount of treated straw consumed daily in period 2 by each animal was considerably less than the amount of untreated straw which it had eaten each day during period 1, but when the untreated straw again took the place of the treated material in period 3, the amount consumed remained practically the same as that eaten during period 2. Considering the apparent zest with which the treated straw was eaten, this is somewhat surprising, but the first decrease can probably be accounted for by the fact that the very large water content of the treated material increased its bulk to such a degree that it was not possible for the animals to consume more, while the

second decrease might have been due to the appetite being adversely affected by the return to the comparatively unpalatable food.

The actual amounts eaten expressed as dry matter were:

Average daily amount of straw consumed (dry matter)

Period	Straw	g.	Horse C		Horse E		Horse D	
			Percentage decrease from preceding period	g.	Per- centage decrease	g.	Per- centage decrease	g.
21 Apr. to 4 June	Untreated	1425.73		1921.06		2519.95		
5 June to 6 Aug.	Treated	1013.44	29.45	1475.16	23.22	2319.73	15.21	
7 Aug. to 3 Sept.	Untreated	1181.65	17.43	1423.90	25.90	2011.73	20.33	

The limitation of stomach capacity for dry matter imposed by the water content of the undried treated food may be important when such material constitutes a large part of the diet.

During the whole period of the experiment the water requirements were noted. When the dry, that is the untreated straw was being fed, no great thirst was shown, in fact the actual amount of water drunk was very small, and although it was offered three times daily, it was only exceptionally that it was accepted more than twice; no water at all was drunk when the wet treated straw was being used, for then the supply in the food would appear to have been overabundant as can be judged by the amount of urine voided.

*Amount of urine voided daily **

Period	Horse C ml.	Horse E ml.	Horse D ml.
When untreated straw used	1516	2213	2026
When treated straw used	2980	4727	7258

Result of the diet on the physical condition of the experimental animals

At the end of the investigation no significant alteration had occurred in the weight of the horses. Their physical condition, however, had undergone pronounced changes. In period 1 the growth of the belly of horses E and D increased, the flanks sagged to a certain extent and, in fact, they assumed the look of unthriftiness associated with animals in store condition. Horse C was already in that state when the work began, but even his general condition deteriorated somewhat during this period.

In period 2 the change in bodily condition was most marked; the belly became a little less prominent, the flanks were fuller, the coat

brighter, and the animals in every way more active and full of life. The change was particularly noticeable in horse C.

There was some slight deterioration again during period 3 but it was not so pronounced as in period 1.

The changes should not be entirely attributed to the food because the improvement, taking place as it did in early summer, coincided with a higher atmosphere temperature. On the other hand, the deterioration in period 3 did not correspond to any marked decline in seasonal temperature.

About half-way through period 2 oedematous swellings appeared on the ventral surface of the belly and breast of horses C and E. They quickly disappeared when the untreated straw was again introduced to the diet, i.e. in period 3. That oedema never occurred in horse D may probably be attributed to the fact that it was the only one that lay down. At all events it would appear that the oedematous condition was associated with an excess intake of water.

Apparently the worm infestation did not appreciably affect nutrition.

SUMMARY

The digestibility coefficients of old barley straw fed with a basal ration of oats, bran and maize to two adult horses and of the same straw after treatment by Beckmann's method were ascertained. The coefficients of the nitrogen-free extractives and the crude fibre of the untreated straw were 33.23 and 38.24 respectively and of the treated straw 54.14 and 63.71. There was a reduction in the digestibility of the small quantity of protein and fat.

Taking into account the 14.5% of dry matter lost during treatment, the starch equivalent value of the treated material was somewhat higher than that of poor quality meadow hay.

The digestibility coefficients were considerably lower for an immature horse previously maintained entirely on fresh grass.

The chemical analysis entailed in this work was carried out by my colleagues Mr Eric Linto and Dr F. E. Moon. To them, and to Dr James Stewart, Animal Diseases Research Association, Moredun Institute, Gilmerton, and to Prof. R. G. Linton, I am indebted for much help and advice.

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SEED DISINFECTION

IV. LOSS OF VITALITY DURING STORAGE OF GRAIN TREATED WITH ORGANO-MERCURY SEED DISINFECTANTS

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(With Plate 9)

INTRODUCTION

THE disinfection of cereal seed with organo-mercury seed dressings has become a general practice in recent years, and so beneficial are the effects that many seed merchants now treat their cereal seed stocks as a routine measure or, alternatively, undertake to treat seed before delivery to their clients. Wakely & Mellor (1938) estimated that in England and Wales alone there were approximately 260 centres where seed treated with these preparations could be obtained, and the number of such centres has undoubtedly increased since then. As bulks of seed may be held for some months after treatment, or even in extreme cases from one season to the next, the probable reaction of such seed in relation to its germination capacity is of importance.

When correctly used organo-mercury seed dressings give an excellent control of many seed-borne diseases, but in some circumstances injury to the grain may result and cases have been reported where considerable loss of vitality has occurred. Weston & Brett (1940) have recorded some factors which predispose the grain to injury: it is the purpose here to deal with these factors more fully and to indicate the conditions necessary to ensure the minimum loss of vitality of treated grain when stored.

MATERIALS

The materials used were well-known proprietary articles described as organic mercurial seed disinfectants. The composition of these powders varies with the particular commercial product, but the main ingredients are very finely divided inert mineral carriers or fillers with which are

mixed or impregnated small quantities of one or more organo-mercury compounds. In some of these the fungicidal substance is often a member of the series $R\text{Hg}X$, where R is a hydrocarbon and X an acidic radicle.

Nature of injury to grain

It has been noted previously (Weston & Booer, 1935; Weston & Brett, 1940) that compounds in this series may produce a characteristic phytocidal effect when they are applied to, and held by, the grain in overdoses. The seed may be killed outright or it may commence to germinate and the coleoptile to appear, but further development is abnormal, characterized by thickening of the tissues of the coleoptile and stunting of the roots. These symptoms, as they apply to wheat, barley and oats, are shown in Pl. 9, fig. 1.

Porter (1936) reports that such seedlings have thickened leaf primordia with irregular crenations and lobes, that cell division is inhibited, the existing cells becoming enlarged and multinucleate either with small nuclei or with large 'giant nuclei' which are polyploid. We have observed that the primary roots are usually short and thickened, and that their development is arrested soon after they emerge. Frequently the root hairs are absent, and in these cases the seminal roots are usually discoloured brown. Typical abnormal seedlings seldom develop further, and neither they nor the killed seeds decay rapidly in the soil. These symptoms are easily diagnosed and are distinct from those caused by the incorrect use of other seed treatments such as copper sulphate, formalin or heat. Injury of these types is shown in Pl. 9, figs. 2-4, where in each case the normal strength of the material used has been deliberately exceeded.

EXPERIMENTAL

Seed dusted at normal and excess rates and stored under ideal conditions

Experiment 1. Small bulks of Wilhelmina, Victor, Yeoman and Little Joss wheats, all of high initial germination capacity, of average moisture content and of sound physical condition, were divided into separate samples and dressed at the rate of 2 and 3 oz. per bushel of seed with two different proprietary organo-mercury seed dressings. Undusted portions were retained as controls. The higher rate of dressing was included in the expectation of obtaining data on the effect of excessive dusting with these dressings. Germination tests were made by the usual routine laboratory method a few days after treatment and also at intervals of 4 months over a period of 2 years. After treatment the

samples were stored in manilla envelopes, under ideal conditions in a laboratory cupboard, and were not subjected to any wide fluctuations of temperature or atmospheric humidity.

When evaluating the significance of the loss of vitality of seed, the major consideration centres around the extent to which such loss renders the seed unsuitable for sowing. For seed to be of reasonably satisfactory cultural value, not only should the total germination be relatively high but the *speed* of germination should be of a high order. It is on this basis that the experimental results are reviewed, but to economize space it has been necessary to omit many tables and to summarize their data.

In the above experiment the results showed that for periods up to 12 months of storage, only those treatments in excess of the recommended rates had any appreciable deleterious effect. After 24 months there was a general tendency for the treatments to reduce slightly both the total and the speed of germination, but again it was chiefly at the overdusting rates that this was in any way pronounced. Up to 12 months after storage, the dusts applied at normal rates caused no greater loss of germination, in the majority of cases, than that shown by the controls. There was also some indication, that one of the dressings, especially at the higher rate of dusting, produced a greater proportion of abnormal seedlings.

Experiment 2. Samples from two distinct bulks of each of the varieties Victor, Wilhelmina, Red Standard and Little Joss were dusted at 2 and 4 oz. per bushel respectively, with the same organo-mercury seed dressings used in the previous experiment, and in each case undusted lots were retained as controls. Although the initial germination of three of the bulks concerned was not high, yet in all eight bulks the seed was of sound physical condition and of average moisture content. Germination tests were made a few days after application of the dusts and at 6 and 12 months later, the seed again being stored in manilla envelopes in the laboratory.

Experiment 3. This experiment was similar to the previous one, except that it included eight small bulks of different varieties of wheat and that germination tests were made at 6, 12, 18 and 26 months after storage.

The results from these three experiments showed that under satisfactory conditions of storage up to 1 year, wheat of good condition, correctly treated, was unlikely to lose vitality to any greater extent than similar seed untreated. When the dusts were applied at rates higher than those recommended in normal practice they induced a more pronounced

loss of vitality, especially if storage was prolonged beyond 1 year. There was a general and progressive increase in the percentage of abnormal seedlings produced over the period of these experiments, a higher proportion of such seedlings occurring when one of the materials was used at the higher dusting rate. Although it was apparent that, in some instances, during storage periods of 12 months or more, some loss of vitality might be expected, yet it was clear that such loss would not be sufficient to give a complete explanation of those cases which had been encountered where rapid deterioration had been experienced, sometimes within a relatively short period after dusting. An investigation was made, therefore, to find to what extent such factors as initial moisture content of the seed and the conditions of storage would influence the loss of vitality of treated seed.

Moisture content of the seed and the conditions of storage

Preliminary tests showed that cereal seed dusted at the normal rate and stored under conditions approximating to maximum humidity rapidly lost germination capacity after the first 4 or 5 weeks; the loss, however, was no greater than with the untreated controls, and ultimately both treated and untreated samples became equally mouldy. This experience was not unusual, as prior to the introduction of the organo-mercury dusts similar results had been noted with formalin-treated seed stored under these conditions. In recent investigations there was also some evidence that the higher the initial germination the lower would be the relative loss of vitality under the given conditions; further, that seed stored at a high relative humidity increased progressively in moisture-content. To obtain further information on some of these points the following experiment was made.

Experiment 4. Bulks of Squarehead's Master wheat, Spratt-Archer barley and Victory oats were each divided into three lots, and in each case the moisture content of two of the subbulks was raised artificially by approximately 2 and 4% respectively. After allowing a reasonable time for equilibrium to be established the moisture content of each subbulk was determined, and then each was further divided to give seven samples of 1 lb. weight. Six samples of each set of seven were dusted separately with three different organo-mercury dressings, A, B, C, at 2 and 4 oz. per bushel. The remaining sample in each set was retained as an undusted control. Each sample of each set of seven was then divided to give two complete sets of six dusted samples and one control. Laboratory germination tests were made upon all the 126 samples at

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Table 1. Shows results of germination tests on samples of wheat of varying initial moisture content, treated with different organo-mercury dusts at different rates and stored under different sets of conditions

Bult, moisture grade and storage conditions	Treatment and rate per bushel	Percentage germination after storage for												18 months					
		2 months				3 months				7 months				10 months					
		6 days	10 days	A.S. days	6 days	10 days	A.S. days	6 days	10 days	A.S. days	6 days	10 days	A.S. days	6 days	10 days	A.S. days	6 days	10 days	A.S. days
Wheat I, Shed	Control	99	99	—	87	90	—	*	*	*	*	*	*	86	—	83	86	—	79
	A at 2 oz.	99	99	0.3	95	97	0.3							93	94	—	90	93	0.6
	A at 4 oz.	98	98	0.3	85	96	0.3							94	95	—	86	91	1.0
	B at 2 oz.	98	98	0.6	91	96	0.3							92	95	2.0	72	87	8.3
	B at 4 oz.	98	98	0.3	54	81	11.6							95	95	1.3	88	97	0.3
	C at 2 oz.	99	99	1.3	88	95	1.6							94	94	—	90	93	0.6
	C at 4 oz.	94	96	2.0	88	93	2.6							95	95	—	86	91	0.6
	Control	98	98	—	93	97	—	85	86	—	86	86	—	93	94	—	83	86	—
	A at 2 oz.	99	99	1.0	96	98	—	—	—	—	—	—	—	94	94	—	90	93	0.6
	A at 4 oz.	95	97	—	88	96	1.3	93	94	—	—	—	—	95	95	—	86	91	1.0
Wheat I, Laboratory	B at 2 oz.	99	99	0.3	95	98	—	94	94	—	—	—	—	92	95	2.0	90	93	0.6
	B at 4 oz.	98	98	0.6	70	91	7.6	92	95	2.0	—	—	—	95	95	—	72	87	8.3
	C at 2 oz.	98	98	—	96	97	0.3	95	95	1.3	—	—	—	95	95	—	88	97	0.3
	C at 4 oz.	96	97	0.6	96	97	0.6	93	93	0.6	94	95	0.6	94	95	1.6	94	95	0.6
	Control	92	95	—	38	39	—	—	—	—	—	—	—	93	94	—	—	—	—
	A at 2 oz.	85	87	1.0	37	38	—	—	—	—	—	—	—	91	91	—	86	91	0.6
	A at 4 oz.	83	90	3.0	42	45	0.6	—	—	—	—	—	—	93	94	—	90	93	0.6
Wheat II, Shed	B at 2 oz.	85	89	1.6	39	40	—	—	—	—	—	—	—	94	95	—	72	87	8.3
	B at 4 oz.	68	85	7.0	36	40	1.6	—	—	—	—	—	—	95	95	—	76	87	7.0
	C at 2 oz.	92	93	0.3	40	40	0.3	—	—	—	—	—	—	93	94	—	88	97	0.3
	C at 4 oz.	86	90	2.6	39	40	0.6	—	—	—	—	—	—	94	95	1.6	94	95	0.6

Wheat I. Initial germination 99%; average initial moisture content 15.74%.
 Wheat II. Initial germination 98%; above average initial moisture content 18.15%.
 Wheat III. Initial germination 95%; high initial moisture content 19.70%.

Wheat II, Laboratory		Wheat III, Shed		Wheat III, Laboratory	
Control	93	97	81	—	36
A at 2 oz.	93	98	55	37	37
A at 4 oz.	85	94	64	43	40
B at 2 oz.	85	98	54	39	32
B at 4 oz.	77	96	1.6	32	34
C at 2 oz.	94	97	0.6	34	34
C at 4 oz.	87	95	2.3	35	35
Control	88	88	—	—	—
A at 2 oz.	83	85	0.6	27	1.0
A at 4 oz.	73	79	2.6	22	2.3
B at 2 oz.	84	87	1.0	22	33
B at 4 oz.	75	81	3.3	18	3.0
C at 2 oz.	87	88	—	32	32
C at 4 oz.	88	89	1.0	25	0.6
Control	85	88	—	53	29
A at 2 oz.	88	90	0.3	42	29
A at 4 oz.	88	90	0.6	64	28
B at 2 oz.	83	84	—	57	27
B at 4 oz.	29	87	1.6	51	28
C at 2 oz.	91	91	0.3	52	29
C at 4 oz.	90	91	1.3	58	32

AS. in five columns above = abnormal seedlings.

- * Samples were rendered valueless by growth of saprophytic fungi.
- † Samples already of no cultural value and no further tests were made.

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Table 2. Shows results of germination tests on samples of barley of varying initial moisture content, treated with different organo-mercury dusts at different rates and stored under different sets of conditions

Barley I. Initial germination 99%; average initial moisture content 15.78%.
 Barley II. Initial germination 99%; above average initial moisture content 17.76%.
 Barley III. Initial germination 99%; high initial moisture content 20.29%.

Percentage germination after storage for

Bulk, moisture grade and storage conditions	Treatment and rate per bushel	2 months						3 months						7 months						10 months						18 months									
		6 days			10 days			A.S. days			6 days			10 days			A.S. days			6 days			10 days			A.S. days			6 days						
		Control	99	99	—	98	98	—	78	79	—	59	61	—	13	13	—	5	5	—	1	1	—	Nil	Nil	—	Nil	Nil	—	—	—	—			
Barley I, Shed	A at 2 oz.	99	99	—	99	99	—	67	67	—	38	38	0.6	13	13	—	5	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—			
	A at 4 oz.	98	98	—	98	99	0.3	52	53	—	28	29	0.6	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			
	B at 2 oz.	99	99	—	99	99	—	60	60	—	4	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			
	B at 4 oz.	99	99	—	95	97	2.3	23	24	0.3	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			
	C at 2 oz.	99	99	—	99	99	—	48	48	—	17	18	0.3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			
	C at 4 oz.	98	98	0.3	98	98	—	53	53	—	21	24	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			
Barley II, Laboratory	Control	99	99	—	99	99	—	96	97	—	96	96	—	96	96	—	96	96	—	96	96	—	96	96	—	96	96	—	96	96	—	96	96		
	A at 2 oz.	99	99	0.3	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99		
	A at 4 oz.	99	99	—	98	98	—	99	99	—	99	99	0.3	97	98	—	98	98	—	98	98	—	98	98	—	98	98	—	98	98	—	98	98		
	B at 2 oz.	99	99	—	99	99	—	97	98	1.0	96	98	0.6	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99		
	B at 4 oz.	99	99	—	99	99	—	99	99	—	97	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99		
	C at 2 oz.	99	99	0.3	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99		
	C at 4 oz.	99	99	—	99	99	—	94	94	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Barley II, Shed	Control	99	99	—	98	98	—	98	98	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99		
	A at 2 oz.	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99		
	A at 4 oz.	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99		
	B at 2 oz.	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99		
	B at 4 oz.	98	98	—	98	98	—	97	97	—	97	97	—	97	97	—	97	97	—	97	97	—	97	97	—	97	97	—	97	97	—	97	97		
	C at 2 oz.	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99	—	99	99		
	C at 4 oz.	99	99	—	99	99	—	94	94	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

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Barley II, Laboratory		Barley III, Shed		Barley III, Laboratory	
Control	99	99	99	99	99
A at 2 oz.	99	99	99	99	99
A at 4 oz.	99	99	99	99	99
B at 2 oz.	99	99	99	99	99
B at 4 oz.	99	99	99	99	99
C at 2 oz.	99	99	98	98	98
C at 4 oz.	99	99	98	98	98
Control	97	97	92	92	92
A at 2 oz.	99	99	94	94	94
A at 4 oz.	99	99	92	92	92
B at 2 oz.	99	99	85	85	85
B at 4 oz.	99	99	90	90	90
C at 2 oz.	99	99	88	88	88
C at 4 oz.	99	99	91	91	91
Control	99	99	96	96	96
A at 2 oz.	99	99	95	95	95
A at 4 oz.	99	99	97	97	97
B at 2 oz.	99	99	95	95	95
B at 4 oz.	99	99	99	99	99
C at 2 oz.	98	98	95	95	95
C at 4 oz.	99	99	98	98	98

A.S. in five columns above = abnormal seedlings.
* Samples were rendered valueless by growth of saprophytic fungi.

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Table 3. Shows results of germination tests on samples of oats of varying initial moisture content, treated with different organo-mercury dusts at different rates and stored under different sets of conditions

Bulk, moisture grade and storage conditions	Treatment and rate per bushel	Percentage germination after storage for																			
		2 months				3 months				7 months				10 months				18 months			
		6 days	10 days	6 days	10 days	A.S.	6 days	10 days	A.S.	6 days	10 days	A.S.	6 days	10 days	A.S.	6 days	10 days	A.S.			
Oats I, Shed	Control	95	95	—	83	84	—	46	47	—	18	18	—	—	—	—	—	—			
	A at 2 oz.	94	94	0.3	91	92	—	—	—	—	27	30	2.0	—	—	—	—	—			
	A at 4 oz.	93	93	0.6	94	96	—	—	—	—	23	26	1.0	—	—	—	—	—			
	B at 2 oz.	94	94	1.0	90	90	1.0	—	—	—	20	20	0.3	—	—	—	—	—			
	B at 4 oz.	95	95	—	91	94	—	—	—	—	39	41	1.3	—	—	—	—	—			
	C at 2 oz.	92	92	1.3	94	94	0.3	—	—	—	24	28	—	—	—	—	—	—			
	C at 4 oz.	93	93	1.3	89	92	1.0	—	—	—	—	—	—	—	—	—	—	—			
Oats I, Laboratory	Control	95	95	—	93	93	—	—	—	—	91	91	—	—	—	—	—	—			
	A at 2 oz.	95	96	0.3	92	93	—	—	—	—	94	96	0.6	93	93	—	—	—	—		
	A at 4 oz.	90	91	1.0	92	93	0.6	93	93	94	—	88	91	0.3	90	91	—	—			
	B at 2 oz.	95	95	0.6	91	92	—	—	—	—	90	93	—	73	92	1.6	91	92	—		
	B at 4 oz.	94	94	0.6	84	89	3.6	—	—	—	91	95	—	90	91	1.3	89	90	—		
	C at 2 oz.	95	95	0.3	90	92	0.3	—	—	—	—	—	—	91	95	—	90	91	—		
	C at 4 oz.	97	97	—	94	94	0.6	—	—	—	—	—	—	91	95	—	90	91	—		

Oats I. Initial germination 95%; average initial moisture content 16.29%.

Oats II. Initial germination 93%; above average initial moisture content 17.48%.

Oats III. Initial germination 90%; high initial moisture content 19.34%.

Oats II, Shed	Control	92	92	—	78	82	—	*	*
	A at 2 oz.	92	0.3	0.3	80	84	0.6		
	A at 4 oz.	91	91	0.3	69	72	0.3		
	B at 2 oz.	91	91	—	71	73	—		
	B at 4 oz.	92	94	1.0	79	81	—		
	C at 2 oz.	92	89	1.0	75	80	—		
	C at 4 oz.	91	92	0.3	75	83	—		
	Control	93	93	—	85	87	—		
	A at 2 oz.	90	90	1.0	86	88	—		
	A at 4 oz.	93	93	0.6	89	91	—		
Oats II, Laboratory	B at 2 oz.	93	93	1.6	80	87	—		
	B at 4 oz.	90	91	1.3	90	92	—		
	C at 2 oz.	92	94	1.0	81	85	2.6		
	C at 4 oz.	95	96	1.0	83	83	—	*	*
	Control	89	91	—	68	69	—		
	A at 2 oz.	89	90	1.3	68	69	—		
Oats III, Shed	A at 4 oz.	87	88	1.0	70	71	1.3		
	B at 2 oz.	86	86	0.6	80	80	—		
	B at 4 oz.	89	90	—	77	78	—		
	C at 2 oz.	89	89	0.6	77	77	—		
	C at 4 oz.	86	88	3.0	61	64	10.6		
	Control	88	88	—	84	85	—		
	A at 2 oz.	91	91	—	85	85	—		
	A at 4 oz.	91	91	0.3	80	81	—		
	B at 2 oz.	90	90	1.3	84	84	—		
	B at 4 oz.	88	90	1.3	83	85	—		
Oats III, Laboratory	C at 2 oz.	91	92	—	86	86	0.3		
	C at 4 oz.	84	90	1.0	73	79	7.0		

A.S. in five columns above = abnormal seedlings.

* Samples were rendered valueless by growth of saprophytic fungi.

† Samples already of no cultural value and no further tests were made.

from 10 to 18 days after dusting, then each sample was placed in a cotton seed bag and one duplicate set of samples was stored in the laboratory, the other in a damp shed. Under both sets of conditions samples of similar original moisture content were stored together. For the first 3 months of the experiment the atmospheric humidity was maintained artificially to prevent any significant fall in the moisture content of the samples. Faulty manipulation of the method adopted actually resulted, in most cases, in an increase of moisture content, but although this fault reduced the value of some of the results, yet the general inferences to be drawn were not affected. Storage was started in February, and there were somewhat wide fluctuations in temperature under the 'shed' conditions of storage throughout the period of the experiment. Accurate measurements of atmospheric humidity were not recorded; the average relative values as between samples in the laboratory and the shed were in the region of 55 and 75 % respectively. Germination tests were made at 2, 3, 7, 10 and 18 months after storage, except in the case of those samples where the incidence of saprophytic fungi made such tests valueless or where germination fell rapidly after the first few months of storage.

The results of these tests are shown in Tables 1-3, and in each case the final germination, the percentage germination at 6 days and the percentage of abnormal seedlings of the type previously referred to is shown.

Moisture-content determinations were made upon the samples at the same time as the germination tests, but those at 18 months were omitted owing to lack of sufficient material. The results of the moisture tests made at the time of application of the dusts showed that for each of the three different cereals included in the experiment the three main subdivisions on the basis of moisture content gave a suitable range from average to very high. In Tables 1-3 these three divisions are indicated as follows:

I = samples of average moisture content.

II = samples above average moisture content.

III = samples of high moisture content.

These results show that treated seed of average moisture content when stored under satisfactory conditions retained its vitality at a reasonably high figure up to 18 months, whereas under conditions of relatively high atmospheric humidity and fluctuating temperature it was of no cultural value after 3 months. There was some indication that the

higher the initial moisture content of the seed, the greater the subsequent germination losses, these being further increased where the seed was stored under definitely adverse conditions. Further, under storage conditions of relatively high humidity, the figures showed that treated seed might lose vitality to a greater extent than untreated seed, but under the conditions of the experiment this was not apparent until at least 3 months after storage. In the majority of cases under good conditions treated samples lost vitality at no greater rate than did untreated seed.

Unused seed from original main bulks (undusted)

When dividing up the three main bulks of varying moisture content, samples of a definite weight were taken and some seed remained unused. This was left undusted and stored in tins with *tightly fitting lids*, and germination tests were made at 2, 7, 10 and 18 months from the commencement of storage. Moisture-content determinations of these samples were also made at the beginning of the experiment and at 7 and 10 months after. In the case of wheat the effect of storing seed of abnormal moisture content in closed containers was very marked at 2 months after storage, and by 7 months neither of the bulks of high moisture content was of any value. The effect was not so pronounced in the case of the barley and oat samples, but the barley II bulk was of doubtful value by 10 months and the barley III valueless by 7 months. The oat II bulk was of little value by 7 months and useless by 10, whilst the oat III was of no value after 7 months. The three bulks of normal moisture content maintained high values throughout the whole period.

The trend of these results emphasized the danger of storing seed of high moisture content in closed containers, and to obtain information as to the effect of storing treated seed in different containers and under different conditions the following experiment was made.

Storage of seed in different types of container

Experiment 5. Separate samples of wheat from a given bulk were dusted with four different proprietary organo-mercury seed disinfectants at the normal rate and a quantity was left undusted as a control. Each portion was further divided to give six samples of 14 lb. each and these were placed in the following containers: two in close-mesh bags, two in open-mesh bags and two in tins with *loosely fitting lids*. One sample from each pair was stored in the laboratory under conditions of even temperature and average relative humidity and the other stored under damp

conditions, the bags being suspended from under the roof of an open shed standing in a damp situation, the tins being kept covered with layers of damp felt and stored in a cool northerly room. A laboratory germination test was made of the original bulk and samples were drawn from each container at intervals throughout the period of storage.

Of the seed stored in bags no significant loss in germination was apparent until 41 weeks after storage, but at 25 weeks there were indications that the seed, in certain instances, was deteriorating. At 41 weeks, losses were noted in those bulks stored under damp conditions and of these those in close-mesh bags suffered more loss than those in the open ones. The control also lost vitality under these conditions, and by 59 weeks had lost considerably more than any of the treated seed. Of the samples stored in bags under dry conditions, none showed signs of serious deterioration even after 85 weeks.

In the case of the bulks stored in tins, the losses were pronounced at a much earlier period, and in two instances there was a considerable loss 10 weeks after the commencement of storage. Losses in all bulks, except the untreated controls and the samples treated with one of the dust disinfectants, were shown by 19 weeks, and the seed which showed marked deterioration at this stage was of no cultural value after 25 weeks. It is particularly interesting to note that the untreated controls maintained a high germination value up to 85 weeks, at which time most of the treated seed was valueless. In this series, the seed stored in tins in the laboratory suffered less than that in the tins stored under damp conditions.

These results showed that the storing of wheat in bags under damp conditions may lead to serious loss of germination within the course of a year, but that seed treated at the recommended rate will lose vitality to no greater extent than untreated seed under the same conditions. Storing in bags under satisfactory dry conditions ensures the maintenance of a high germination value for well over a year, but storage of treated seed in closed containers may lead to serious loss of germination capacity by 3 months and the seed may be valueless by 6 months.

The quantity of dust dressing retained by wheat in sound condition and superficially dry

Experiment 6. From previous experience it was known that although the various proprietary organo-mercury seed dressings which had been used in the experiments were all held by grain at the recommended rates of application, yet there was divergence between them in relation

to the maximum quantity which could be held by dry seed in good condition. As it was of interest to determine to what extent over-dusting could be effected, experiments were made with four proprietary organo-mercury dusts to find the maximum quantity, in each case, which could be held by wheat in good sound condition and superficially dry. These results are shown in Table 4, but the figures should be accepted as approximate only, as these values may vary slightly with different wheat samples, the container used for storing the dust and the existing meteorological conditions. The retention value of the dust itself will depend upon the type of filler, its state of subdivision and whether or not substances of an adhesive nature have been added.

Table 4. *Shows the maximum retention values of four proprietary organo-mercury seed dressings*

Material	Maximum quantity retained by wheat in good condition, oz. per bushel
W	5
X	7
Y	6
Z	2½

It will be seen that there is considerable variation amongst these different materials in the maximum amounts which can be held by a well-conditioned sample of seed wheat, a feature of particular importance in relation to over-dusting and especially to wheat treated when it is superficially damp or in an otherwise unsuitable condition. It must not be assumed, however, that because the maximum retention value of one dust is greater than that of another this necessarily implies that the former dust will be more toxic, for the phytocidal and fungicidal effects will depend primarily on the nature and quantity of the organo-mercury compound which is contained in the dressing. It is likely that a seed disinfectant with a high retention value and containing salts of high toxicity, especially if applied in over-doses, will be more liable to reduce germination capacity of a badly conditioned sample or one stored under adverse conditions.

The dusting of dry and moist grain at excessive rates

Experiment 7. A stock of Red Marvel wheat was divided and one half moistened so that the surface of the grain was moist to the touch. Samples from this were dusted with a proprietary organo-mercury seed disinfectant at the rate of 2, 4, 6, 8 and 10 oz. per bushel. The other

half, the dry seed, was also sampled and portions treated with the same material at 2, 4, 6 and 8 oz. per bushel. In both cases undusted seed was retained, all samples being placed in manilla envelopes and germination tests started 5 days later. The results of these tests are shown in Table 5, where, in addition to the final germination figure, the interim germination counts are also shown, together with the percentage occurrence of abnormal seedlings. For a period up to 10 days seedlings of an abnormal character were not removed at the interim germination counts but were left on the seed beds. At the final count those which had assumed an appearance of normality in the intervening period were then counted and included amongst the normal seedlings removed. This explains the differences in 1 day—the 10- and 11-day counts—in the case of the moist seed dusted at 8 and 10 oz.

The dressing used was known to be held by wheat in normal condition at a maximum rate of approximately 7 oz. per bushel, this quantity being retained by the seed provided it was not agitated.

It is probable that the majority of the dry seed dusted at 8 oz. would have held the dust at a rate not greater than 6 oz. per bushel by the time that the seed was planted in the seed beds. The moist seed retained the dust at 10 oz. without any excess, and no loss of dust was noted until the seed dried and then only when it was disturbed.

Table 5. Shows the results of germination tests made upon seed wheat of sound dry condition treated with a proprietary organo-mercury dressing at varying rates, compared with tests upon superficially moist seed of the same bulk, treated in the same manner

Condition of seed when dusted	Rate of application of the seed dressing, oz. per bushel	Percentage germination in				Percentage of abnormal seedlings
		4 days	6 days	10 days	11 days	
Superficially dry	Control	97	99	99	99	—
	2	97	98	98	98	—
	4	97	98	98	98	0.6
	6	96	99	99	99	0.3
	8	96	98	98	98	0.3
	10	28	41	58	83	15.3
Superficially moist	Control	95	96	96	96	—
	2	87	95	98	98	—
	4	83	90	98	98	1.3
	6	68	81	95	95	4.0
	8	39	53	71	87	6.3
	10	28	41	58	83	15.3

This experiment showed that superficially moist seed retained a dust disinfectant at rates considerably in excess of those recommended, and that when it was applied in overdoses the material exerted a greater

phytocidal effect than when applied to dry seed in similar amounts. A feature of particular interest was the *retarding* effect upon germination induced by the dust upon moist seed, the effect being a progressive one with increased rates of dusting. It should be noted, too, that at the higher rates of dusting moist seed the total germination was reduced, and this is in marked contrast to dry seed where, for all practical purposes, the effects were negligible. It must be emphasized, however, that these tests were made within a few days after treatment of the seed and not after lengthy periods of storage.

DISCUSSION AND SUMMARY

Failures of cereal crops are occasionally reported, the seed having been dressed with an organo-mercury seed disinfectant and the failures attributed to the dressing. It should be noted, however, that prior to the introduction of the organo-mercury seed dressings similar crop failures had often been recorded, and in many cases failure had been attributed to the dressing which had been applied, or alternatively, to the conditions under which the treated grain had been stored.

Reduced germination during storage is common to all agricultural and horticultural seeds, but although the interaction of the factors involved is complex it is generally recognized that for satisfactory germination to be maintained over the maximum period of storage the following conditions should be observed:

- (1) The seed should be of relatively high initial germination.
- (2) The moisture content of the seed should approximate to the average for that kind.
- (3) Storage temperature and humidity should both be relatively low and *not* subject to wide fluctuation.
- (4) Ventilation should be adequate.

The experiments described confirm these general principles in relation both to untreated seed and to grain treated with organo-mercury seed disinfectants. The majority of the experiments recorded here were confined to wheat and showed that seed of high initial germination, average moisture content, sound physical condition, dusted as recommended and stored under satisfactory conditions, did not lose vitality to any greater extent than untreated seed, during at least 1 year's storage. In some cases a high level of germination was maintained over a longer period. These results applied to seed stored both in envelopes and in jute bags. It was noted that where the dust disinfectants were

applied in over-doses they induced a more pronounced loss; especially after a year's storage.

Trials with wheat, barley and oats showed that the moisture content of the seed and the conditions of storage materially influenced losses, conditions of relatively high humidity and of fluctuating temperature leading to rapid loss both in the case of treated and untreated seed. High moisture content of the seed in conjunction with these adverse conditions led to even more rapid loss of germination. A difference was noted in the behaviour of wheat, oats and barley under adverse storage conditions, wheat losing germination more rapidly than oats and oats more rapidly than barley. Further, the phytocidal effect of the organo-mercury dusts was more pronounced in the case of wheat, less in oats and least in barley. This difference may be correlated with the botanical features of the grain. In threshed wheat the caryopsis is free from the paleae, in oats it is free from the paleae but the latter more or less tightly envelop it, whereas in barley the caryopsis is firmly united with the enclosing paleae.

Storage of treated seed wheat in closed containers led to very rapid loss of germination, whereas untreated seed under the same conditions retained high germination for well over a year.

A feature of considerable importance in relation to over-dosing was the variation in the maximum amount of the different proprietary dust disinfectants which could be held by well-conditioned grain.

Superficially moist seed retained dust disinfectants at rates much in excess of those recommended. When applied to such seed in over-doses these materials led to retardation and reduction of germination and, in addition, the phytocidal effects were materially increased.

Under practical conditions of dusting grain it is possible that if superficially moist seed is treated, even at the recommended rate, part of the bulk of seed upon which the dressing falls will retain excessive quantities. As the retention value of moist seed is high, the subsequent mixing may not result in an even distribution of the powder but in a distribution throughout the bulk of a proportion of heavily over-dusted seeds. These may be killed or produce abnormal seedlings, thus resulting in an uneven stand. Intentional or accidental application of dust to superficially moist seed, at rates higher than those recommended, would tend to produce more markedly adverse results.

It should be emphasized that as only some proprietary seed disinfectants of the organo-mercury type were included in these experiments, the results should be interpreted as a general indication of the possible behaviour under certain conditions of seed treated with such materials.

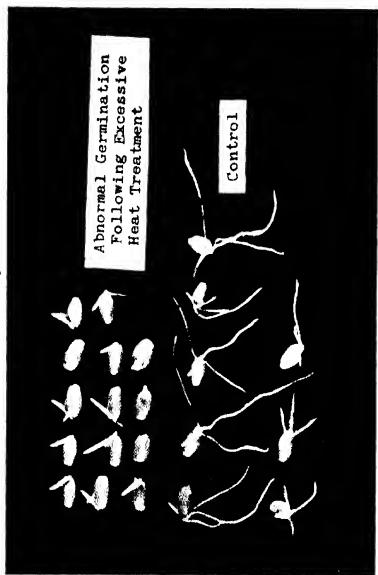


Fig. 1

Fig. 2

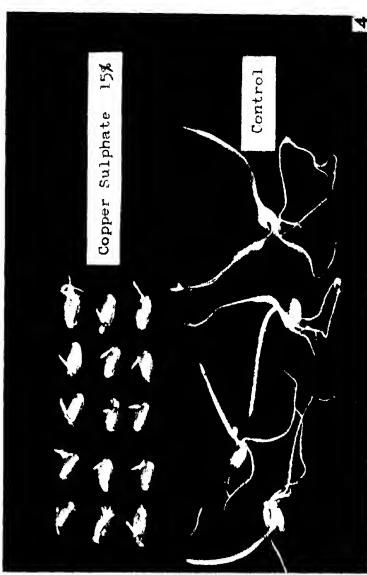


Fig. 3

Fig. 4

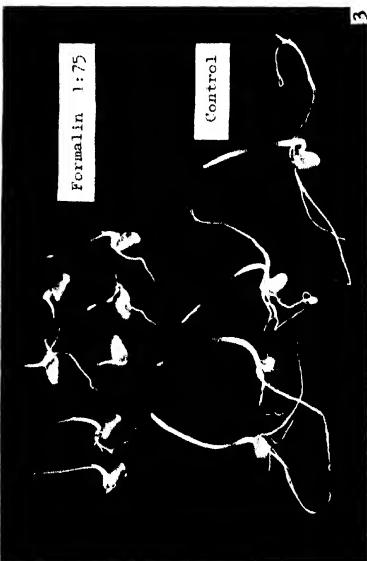


Fig. 4

We are most indebted to the Chief Officer of the Official Seed Testing Station, Mr A. Eastham, for granting us every facility for this work and also for his sustained interest during the course of the investigation. We thank Dr R. E. Taylor for the preparation of the photographs.

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EXPLANATION OF PLATE 9

Figs. 1-4. Types of injury caused by various seed treatments when these have been incorrectly used.

Fig. 1. Injury by organo-mercury dusts: the tissues of the coleoptile are thickened and the roots stunted.
Fig. 2. Injury by excessive heat treatment: root development is completely arrested, the coleoptile being relatively normal.
Fig. 3. Injury by formalin: the development of plumule and roots is only partially arrested.
Fig. 4. Injury by copper sulphate: the development of both plumule and roots almost completely arrested.

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NOTE ON 'THE SIGNIFICANCE OF THE *pH* DETERMINATION IN THE EVALUATION OF *QUALITY* IN SILAGES'

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It is now generally recognized that the *pH* of a silage is the best single criterion of its *quality*. In other words, it is regarded as the best single guide to the type of fermentation that has taken place in the mass in the silo and thus serves to indicate the degree of success achieved in the ensilage process, whatever the crop or its stage of growth. For instance, in the case of silages, made with or without molasses, where the *pH* is at or below 4.0, the silage would be deemed to be of 'excellent' quality, that at 4.0-4.5 would be designated 'good', that at 4.5-5.0 only 'moderate', and at or above the latter figure the silage would be classified as 'bad'. To get a complete picture of the chemical changes that have taken place and to assess the *feeding value* of a silage is quite a different matter and is not the object of the present note.

In the course of work on silages made in 1940, it became increasingly clear that the *pH* figures for 'overheated silages' were of doubtful value. These seemed to vary with the degree of overheating—not an easy matter to assess—and often values were obtained in the region of 4.0 or below, and between 4.0 and 4.5, which would entitle them, on the above assumption, to be classified as 'excellent', or 'good', respectively.

Two special instances served to concentrate the writer's attention on the matter. One was a dark brown oat and pea silage with pleasant smell and obviously extensively overheated and accompanied by a considerable amount of waste inedible material at the sides and top of the silo. This silage had a dry-matter content of 44.8% and had a *pH* of 3.2—a value far below any previously met with and out of the range of *pH* values for silage made without added acid. The other was a very dark brown silage made from moderately mature grass, had a pleasant smell and was associated with a large amount of waste inedible material. The dry-matter content of this silage was 36.5% and the *pH* 3.78—a figure not often obtained with molassed silage.

To investigate this matter, a stack silage, made from a mature crop

of meadow grass intended for hay and ensiled in July, was selected for the purpose. An examination in the field revealed that the bulk of this stack was very definitely overheated. The extreme top was not available at the date of the examination, but the remainder presented the following picture: a layer of dry mouldy material at the top followed by a mass of brown material which had overheated to different degrees and finally, at the bottom, a layer about 1 ft. in depth of yellowish brown material with pleasant smell. A sample taken from the centre of the bottom layer had a dry-matter content of 22.3%, a crude protein in the dry matter of 11.3% and a pH of 4.0, and looked a normally well-made silage.

Three samples taken from the definitely overheated layers had the following characteristics:

(a) Very dark brown, pH 3.82	Smell like that of overheated hay.
(b) Very brown, pH 4.07	
(c) Brown, pH 4.31	

In order to verify the inference indicated by the foregoing figures, namely, that overheated silages have pH values varying with the degree of overheating, the contents of a second silo were investigated.

In this case, the silage was made from moderately mature meadow grass in an improvised silo constructed from old corrugated iron sheets (approx. 16 ft. in diameter and 10 ft. high) with the corrugations running horizontally. A large amount of waste inedible material was evident—about 15 in. all round the sides and 10 in. at the top. The bulk of the mass showed up in definite layers, indicating stages of overheating. An examination of samples taken from these layers gave the following results:

- (a) Mouldy brownish black slimy layer at top, pH 8.06.
- (b) Waste material at sides, pH 7.55.
- (c) Very dark brown layer with practically no smell situated 1 ft. from top, pH 4.10.
- (d) Dark brown to blackish layer, no smell, material disintegrated, situated 3 ft. from top, pH 3.89.
- (e) Brown layer with pleasant tobacco smell, situated 4 ft. from top, pH 4.32.

The dry matter of sample (e) was 26.6% and the crude protein in the dry matter 9.9%.

Before proceeding further, the method of determining the pH may be given.

About 70 ml. of juice was obtained from each sample by a press. The well-mixed extract was then used for the determination with a quinhydrone electrode and potentiometer. Duplicate determinations were made in all cases after making absolutely sure by means of a buffer solution that the instrument was giving correct readings.

It seems clear from the above results that silages with *pH* values in the neighbourhood of 4.0 may or may not be of 'excellent' quality. Grossly overheated silages, however, which have *pH* values of this order are not likely to be misinterpreted, as this degree of overheating is manifest even to the untrained eye. It is, on the other hand, quite a different proposition to detect silages that have overheated to a less pronounced extent unless they have been examined *in situ*.

The following silages,¹ whose dry-matter contents and *pH* values are given below, were definitely overheated as judged by colour, smell and the general conditions obtaining in the silo.

Dry matter		<i>pH</i>	Colour
No.	%		
28	30.5	4.10	Yellowish-brown
4	30.5	4.20	Brown
E	31.7	4.24	Brown
15	30.8	4.30	Yellowish brown
F	32.5	4.30	Light brown
37	26.5	4.36	Light brown
9	29.0	4.43	Brown
33	27.6	4.43	Brown
16	39.0	4.43	Yellowish brown
41	31.1	4.45	Yellowish brown
32	35.2	4.46	Yellowish brown
40	33.2	4.46	Brown
31	37.3	4.60	Light brown
7	29.6	4.64	Brown
24	29.1	4.68	Brown

On the basis of the *pH* classification stated previously, the first twelve silages above would normally be grouped as 'good', and the last three as 'moderate'. By so doing, their feeding value as deduced from other analytical figures would undoubtedly be overestimated.

When silages are examined in the laboratory without any reference whatever to the conditions obtaining in the silo, overheating, unless very pronounced, may easily be overlooked. This, coupled with the fact that the *pH*-value may be within the range of well-made silages, is likely to lead to a misinterpretation of their feeding value—the ultimate end in view.

From the evidence submitted, it would seem that the *pH* is of little assistance in the evaluation of *quality* in silages except in special cases.

¹ All grass or grass and clover except No. 4 (oat and vetch) and No. 41 (oat and pea).

No discrimination can be made by means of it between 'well-made' and 'overheated' silages. Again, so-called 'sour' silages can easily be recognized by the smell of butyric acid.

One special case may be mentioned. A very strongly acetic silage may possibly be confused with a 'butyric' silage if the *pH* value is not available, and one's sense of smell is not acute.

In the writer's view, *colour*, *smell* and *dry-matter* content are the best characteristics of *quality* in a silage as apart from *feeding value*. The *pH* values, especially in overheated silages, have been shown from the above results to be misleading.

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